THE UNIVERSITY OF ALBERTA

HUMIDITY BEHAVIOR AND RECEPTION IN THE SPHAEROMID ISOPOD,

GNORIMOSPHAEROMA OREGONENSIS (DANA)

by

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A THESIS

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The undersigned certify that they have read, and
recommend to the Faculty of Graduate Studies and Research, for
acceptance, a thesis entitled "Humidity behavior and reception
in the sphaeromid isopod, Gnorimosphaeroma oregonensis (Dana)"
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# ABSTRACT

This study was undertaken to examine the humidity behavior of the sphaeromid isopod, _Gnorimosphaeroma oregonensis_, and to test the hypothesis of an osmotic or ionic mechanism of humidity reception. This intertidal isopod often inhabits air exposed, moist microhabitats with high relative humidities. Movements away from these microhabitats are probably prevented by orientation mechanisms. _G. oregonensis_ orients behaviorally in an atmospheric humidity gradient. In a gradient of 76% RH to 100% RH at 20°C, aggregation in high humidities is effected by a reversal turn reaction.

In testing the hypothesis of an internal osmotic or ionic mechanism of humidity reception with a salinity acclimation approach, it was found that activity rates and response intensities are affected by hemolymph concentrations in _G. oregonensis_. These results suggest that humidity information may be received via receptor cells or neurons that are sensitive to changes in the osmotic concentration of the hemolymph or in the concentration of a specific ionic component of the hemolymph. An evaporative cooling mechanism for the reception of humidity information may also occur in _G. oregonensis_.

# ACKNOWLEDGEMENTS

TABLE OF CONTENTS

# LIST OF TABLES

LIST OF APPENDIX FIGURES

LIST OF APPENDIX FIGURES (Continued)

INTRODUCTION

Orientation behavior in various humidity conditions has been
analyzed and described in isopods by Gunn (1937), Waloff (1941),
Fraenkel and Gunn (1961), Lagerspetz and Lehtonen (1961), and Perttunen
(1961) and in amphipods by Williamson (1951) and Lagerspetz (1963).
Concurrently, the isopod studies of Miller (1938), Brooks (1942),
Cloudsley-Thompson (1952, 1956), Den Boer (1961), Warburg (1964), and
Lindqvist (1968) have approached humidity behavior problems by empha-
sizing activity patterns, interactions of ecological factors, or both.
With the exception of the brief paper by Lagerspetz and Lehtonen (1961)
on Asellus aquaticus and Idotea baltica, all of the isopod work on
humidity behavior has been accomplished on oniscoid isopods. Further-
more, most of the oniscoid studies have been limited to three wide-spread
species: Oniscus asellus, Porcellio scaber, and Armadillidium vulgare.
To provide more comparative information on humidity behavior, the
present study focuses attention on the intertidal sphaeromid isopod,
Gnorimosphaeroma oregonensis (Dana).

In isopods and amphipods a number of attempts to demonstrate the
presence of and the localities of specific external hygroreceptors have
been reported. Using histological techniques, Jans and Ross (1963)
discovered pits beneath the thoracic lateral plates of a few oniscoids
which they tentatively believed to be thermo- or hygroreceptors; how-
ever, their observations lack experimental confirmation. Indirect
experimental attempts involving the removal of and the masking of
appendages or other areas of the body have failed to provide any

positive evidence for the existence of specific external hygroreceptors (Gunn, 1937; Miller, 1938; Janda and Lang, 1939; Williamson, 1951; Cloudsley-Thompson, 1956; and Lagerspetz and Lehtonen, 1961). To date no one has undertaken an electrophysiological approach in isopods or amphipods.

Because some isopods, including G. oregonensis, orient in humidity gradients and because most of the evidence does not support the existence of specific external hygroreceptors, mechanisms for indirectly receiving humidity information become reasonable alternative hypotheses. Indirect mechanisms, functioning evaporimetrically, might involve cellular sensitivity to chemical, osmotic, mechanical, or thermal changes (Pielou, 1940).

There are at least three ways to behaviorally test the hypothesis of an indirect osmotic or ionic mechanism. First, experimental animals could be desiccated in air and compared with normally hydrated controls in humidity gradients. The hemolymph of the former animals would concentrate more than that of the latter animals (Widmann, 1936; Parry, 1953; Bursell, 1955; Wilson, 1970), and the humidity behavior of the experimental animals might differ from those of the control animals. Although applicable to oniscoids, this approach suffers from the disadvantage that cuticular permeability and hydration decrease in desiccated animals, but not in hydrated animals (Bursell, 1955). Consequently, differences in cuticular structure and function between the desiccated and the hydrated animals would probably modify humidity behavior and confound the results.

The second way to test the hypothesis of an osmotic or ionic mechanism would be to either withdraw hemolymph from or to inject different concentrations of ion solutions or hemolymph into the

hemocoele of normally hydrated animals. Experimental and control

animals could then be compared in humidity gradients. Difficulties

would arise, however, in avoiding hemolymph volume changes.

Finally, the hypothesis could be tested by acclimating osmocon-

forming animals that are tolerant of a wide range of salinities to

different concentrations of sea water and by subsequently testing the

animals in humidity gradients. If humidity information influences

behavior through osmotic or ionic mechanisms, animals acclimated to

high and to low salinities should behave differently in humidity

gradients. Because most oniscoids tolerate wide ranges of sea water

briefly (Brusca, 1966), the salinity acclimation approach herein

reported was accomplished instead with the euryhaline sphaeromid,

G. oregonensis.

Thus, this study attempts to describe the humidity behavior of

G. oregonensis and to test the hypothesis of an osmotic or ionic

mechanism of humidity reception. This isopod is especially appropriate

for this study because it responds to humidity conditions, it tolerates

wide salinity ranges, and it approaches hemolymph osmoconformity.

MATERIALS AND METHODS

In addition to temperature and humidity measurements, field behavior and habitat observations of G. oregonensis were recorded from localities from Port Renfrew, British Columbia to San Francisco Bay, California. The areas most intensively studied were Albert Head Lagoon, near Victoria, British Columbia; Willapa Bay in Pacific County, Washington; and Tomales Bay in Marin County, California. Field humidity data were taken with a pistol-type Atkins wet bulb-dry bulb hygrometer that facilitated measurements from the various microhabitats of G. oregonensis.

For the behavior experiments, G. oregonensis were collected from Albert Head Lagoon, located 3.6 miles south of Colwood, British Columbia. The animals were maintained at the University of Alberta in 75% (100% = 29.7 $^{o}$/oo) Instant Ocean sea water (Aquarium Systems; Wickliffe, Ohio) at 21°C.

Because G. oregonensis is euryhaline and is a fairly good osmo-conformer to a wide range of test salinities in the first 48 hours of exposure (Riegel, 1959a), animals were acclimated to either 100% or 25% Instant Ocean sea water for 48 hours at 20°C prior to testing in humidity gradients. According to Riegel's Figure 2, the maintenance salinity of 75% sea water is near the isosmotic point of G. oregonensis. In the 25% acclimation salinity, G. oregonensis hyperregulates slightly to a hemolymph concentration of about 28% sea water (100% = 29.7 $^{o}$/oo), and in the 100% acclimation salinity, G. oregonensis hyporegulates slightly to a hemolymph concentration of about 70% sea water. Hence, after 48 hours the hemolymph of animals acclimated to 100% sea water

is about 2.5 times as concentrated as the hemolymph of animals acclimater to 25% sea water.

The humidity behavior of G. oregonensis was tested in modified alternative chambers (Gunn and Kennedy, 1936; Perttunen, 1953). Each chamber consisted of two circular plexiglass lids with internal diameters of 20 cm and internal depths of 1.6 cm (Fig. 1). One lid was inverted and connected around its circumference to the other by a rebated joint. The bottom lid was divided into three troughs by two parallel partitions 8 cm apart and 6 cm from the outer edges. One extreme trough was filled with distilled water and the other with saturated NaCl solution to establish a theoretical humidity gradient of 100% to 76% relative humidity (RH) at 20°C. Control chambers were filled in all troughs with a saturated $K_2Cr_2O_7$ solution to produce a constant relative humidity of 98.0% at 20°C (high humidity controls) or with a saturated NaCl solution to yield a constant 76.0% RH at 20 C (low humidity controls) (Winston and Bates, 1960). Actual relative humidities probably differed little from theoretical values because chambers were reused only after reequilibrating for 24 hours, salt solutions were well separated in the gradient chambers, and temperatures varied ≤ 1°C. However, attempts to measure actual relative humidity values by the cobalt thiocyanate colorimetric method (Tintometer, Ltd., Salisbury, England; Solomon, 1957) yielded low values because the chambers did not completely re-equilibrate in the two hour exposure time specified by this method (Fig. 2).

The top lids of the chambers were perforated by nine small tightly stoppered holes, one in the middle for the introduction of animals and eight equally spaced peripherally for the removal of animals without

Figure 1. A modified alternative chamber with a theoretical humidity gradient of 76% relative humidity (RH) to 100% RH at 20°C

*G. oregonensis*

behavior platform
with centimeter grid

stoppers

plexiglass
lids

distilled
water
(100% R.H.)

saturated NaCl
(76% R.H.)

partitions
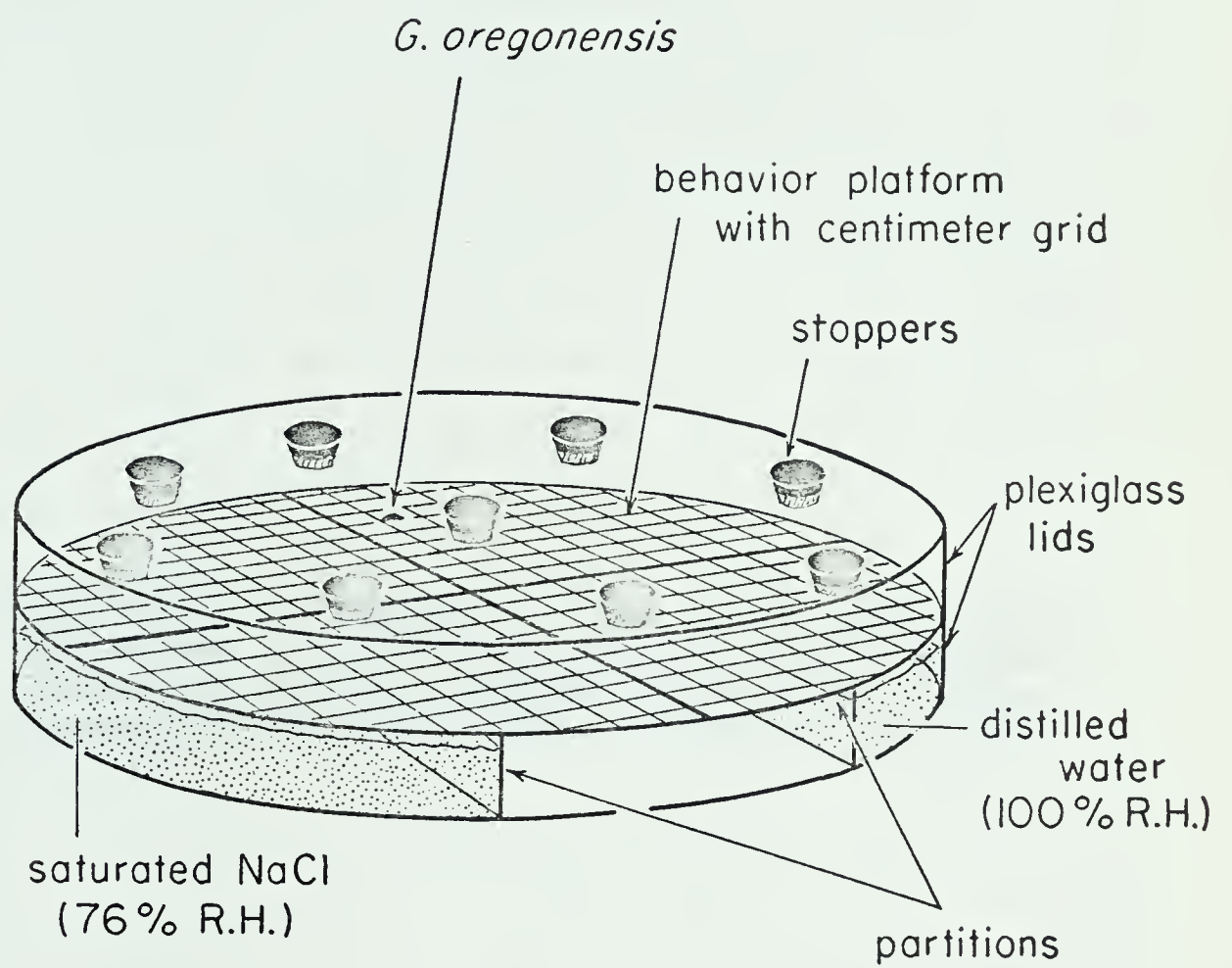
Figure 2.  Mean position frequencies at each chamber position of
100% SW and 25% SW acclimated gradient tested animals.
Theoretical and measured relative humidities (RH) and
theoretical saturation deficiency (SD) are also shown.
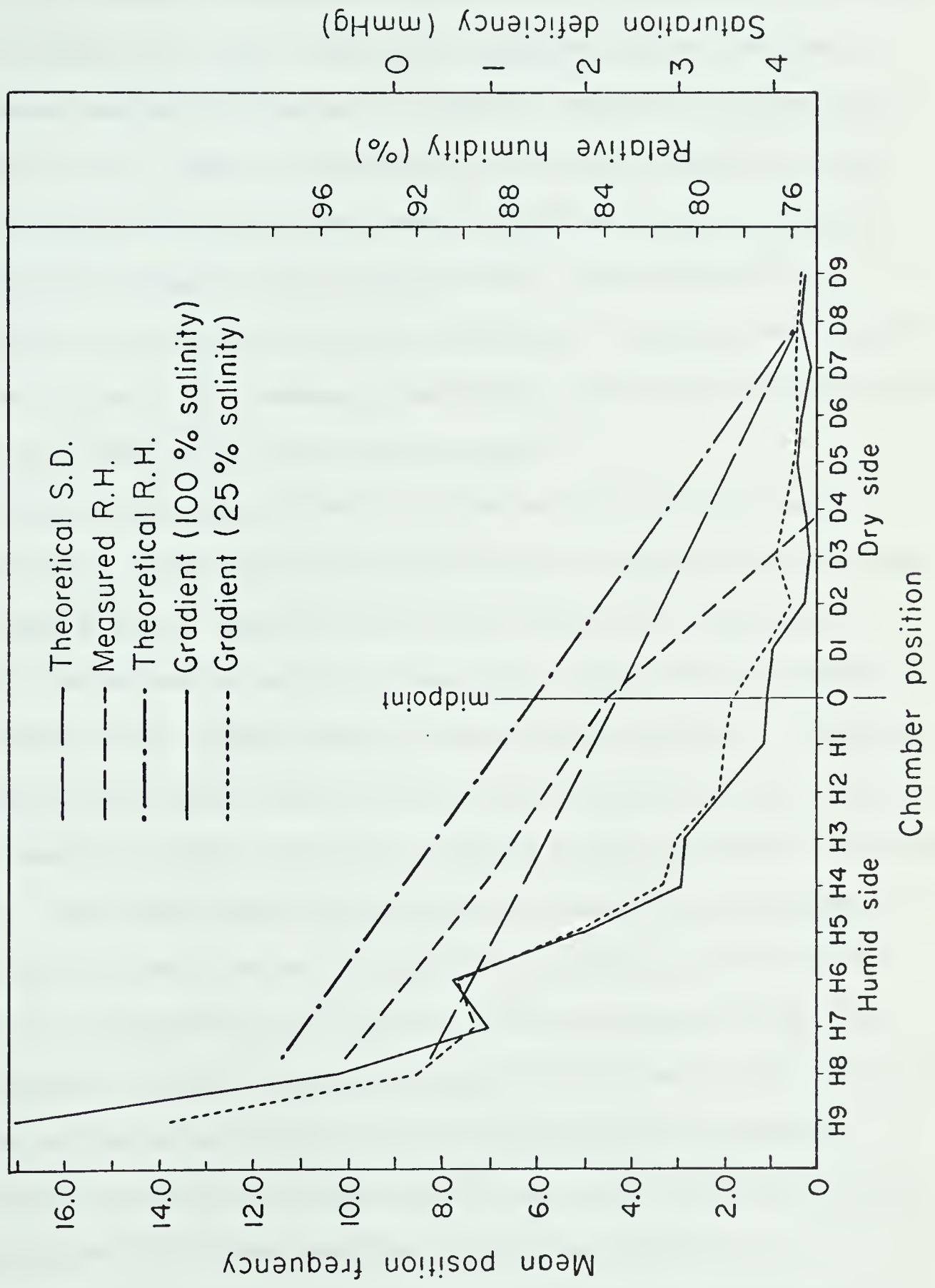
markedly disturbing the humidity conditions in the chambers. Between the two lids a tightly woven (22 meshes/cm × 33 meshes/cm) piece of black nylon fabric, the behavior platform, was stretched and held taut by the interlocking lids. Penciled centimeter grids on the fabric platforms provided coordinates for defining positions of animals at points in time. Fine wire screen platforms were unsuitable because abnormal behavior resulted when the pereipods of animals protruded through and sometimes caught in the meshes. The platforms were situated 0.4 cm above the salt solutions and 1.2 cm below the top lid. The two halves of the chambers were tightly sealed together with plastic electrical tape and silicone rubber sealant.

After acclimating to 100% or to 25% sea water for 48 hours, animals were allowed to self-blot on damp filter paper in a petri dish for three minutes. Single animals were placed in an alternative chamber and allowed to adapt for one minute. Thereafter, positions of the animal in the chamber were recorded every 0.5 minutes. A manually operated multi-channel event recorder was used to record numbers and time lengths of stops, numbers of ordinary turns, and numbers of reversal turns. Very brief stops of 2.5 seconds or less were not recorded. An ordinary turn was a 90° to 180° change in direction anywhere in the chamber accomplished within a moving distance of no more than 5 cm. In contrast a reversal turn was an abrupt turn of about 180° within 4 cm of the midline which usually reversed the direction of movement. Reversal turns were most often observed when an animal was moving from the more humid half into the dryer half of the gradient.

All of the behavior experiments were accomplished on single active adult female animals 4 to 5 mm long in a constant temperature

room at 20° to 21°C. Observations were made under a 200 watt heat-shielded light bulb with a red filter that was suspended four feet above the chambers. Chambers were rotated half way through the experiments. Experiments were run from 22.00 hr to 01.00 hr MST from July to December, 1969. For each animal data were recorded for 30 minutes, and individual animals were never rerun.

For the evaporative water loss experiment, G. oregonensis were collected from San Francisco Bay, California, maintained in 75% sea water at 18°C, and acclimated to either 100% or 25% sea water at 18°C for 48 hours prior to testing. After a one minute self-blotting on damp filter paper that removed the surface water film, adult female animals were initially weighed in small plastic capsules on a Mettler M5 microbalance to the nearest 0.001 mg. After exposure for one hour to moving air having a flow rate of 186 ml/min and a humidity varying from 70% to 80% RH at 18°C ±1°C in an apparatus similar to Edney's (1951a) separator tube, animals were reweighed as before. Animals acclimated to 100% or 25% sea water were run simultaneously and placed alternately in successive chambers of the separator tube. Percent weight losses were calculated for each animal; water evaporation constituted all of the weight loss.

Significance tests were run between the conditions in the following groups: (1) 100% sea water (SW) acclimated animals in gradients and 25% SW acclimated animals in gradients, (2) 25% SW acclimated animals in gradients and 25% SW acclimated animals in constant 98% RH (high humidity controls), (3) 25% SW acclimated animals in gradients and 25% SW acclimated animals in constant 76% RH (low humidity controls), and (4) high humidity controls and low humidity controls. Also, some tests

were run within each experimental group on data from the first 15 minutes and the second 15 minutes of the experimental period or on data from the more humid side (87% to 98% RH) and the dryer side (76% to 87% RH) of the experimental chambers. Because animals in the gradient conditions spent less time in the dry side than in the humid side, some data (i.e., numbers of stops and all of the turn data) were not directly comparable between the two sides when applied to kineses questions. These data were multiplied by correction factors to adjust the data to equal time periods spent in each side. For the evaporative water loss experiment, 100% SW acclimated animals were compared with 25% SW acclimated animals. Parametric data (i.e., position proportion, activity, speed, stop time, and evaporative water loss) were compared with single classification analysis of variance tests (F); prior to this test, position proportion data and percent weight loss data underwent an arc sine transformation. Adjusted Chi-squares ($\chi^2$) were calculated to compare the number of position records on the two sides of the chamber in each condition. Stop numbers and all of the turn data, however, were analyzed with Wilcoxon two-sample tests ($t_s$ and $U_s$). Significance levels of all of the tests were set at the 95% probability level, and all of the statistical tests were accomplished as outlined in Sokal and Rohlf (1969). Mean position proportion data were the number of position records on the humid side divided by the number of records on both sides. "Mean excess percentages" (MEP) of the position data were calculated by the formula:

$$MEP = \frac{\text{number humid side positions} - \text{number dry side positions}}{\text{total number positions}} \times 100$$

(Bentley, 1944).

Mean excess percentages can vary from +100% to -100%. Positive percentages result when more time is spent in high humidity conditions than in low humidity conditions and _vice_ _versa_. Both mean position proportions and mean excess percentages are measures of response intensities. The mean minimum activity of an animal is the mean straight-line distance between position records in cm/min. Numbers of stops and stop times influence mean minimum activities. Mean minimum speed is also the mean straightline distance between positions; however, stop data are subtracted from the calculations. Since animals rarely moved very far in a straight line, these measures of activity and speed are comparable minimums.

RESULTS

Field Observations and Measurements

G. oregonensis is distributed from Alaska to central California

and Hawaii (Richardson, 1905; Hatch, 1947; Menzies, 1954; Riegel, 1959a,

1959b). Although often collected under or around rocks or mussel beds

in variously brackish bays, the animal has been taken from protected

outer coast situations (Menzies, 1954), from under mussel crusts on

gravel shores (Ricketts and Calvin, 1968), from depths of 10 to 12

fathoms (Hatch, 1947), from open water near submerged night lights

(Hatch, 1947; Menzies, 1954), and in salinities as low as about 9 $^o$/oo

(Menzies, 1954). The writer has collected this common isopod from

crevices and surfaces of rocks, logs, boards, styrofoam, slabs of bark,

mussels, and other rough  stable substrata in bays, estuaries, lagoons,

and other protected coastal situations from British Columbia to central

California.

Generally, the behavior of G. oregonensis resembles that of onis-

coid isopods. When exposed to air, animals are usually clamped firmly

to rough objects; however, in water this rugophilic behavior is occasion-

ally displaced by relatively slow crawling or fast swimming responses.

Like many oniscoids, G. oregonensis is usually seen in scattered

aggregates of from a few to many hundreds of individuals. Furthermore,

G. oregonensis displays a positive geotaxis and a negative light response,

probably a photo-tropo-taxis, together with a response towards high

humidities. Although the latter is the major concern here, complex

interacting responses to contact, slope, light, temperature, and humidity

conditions determine behavioral responses in natural environments.

G. oregonensis is often found in air immediately along the water line and up to several feet out of the water, usually under damp rocks or logs. In these situations animals are often inactive, and their moist cuticles suggest that the animals are well hydrated. Some less frequently encountered, albeit interesting, examples are documented here. In May, 1969, conglobating G. oregonensis were uncovered along with spiders, millipedes, insects, and several different oniscoids beneath moist logs as far as 20 feet from the water line in nontidal Earl Lake in Del Norte County, California. At Big Lagoon in Humbolt County, California, a single torpid and partially desiccated conglobating animal was found nestled in slightly moist gravel under a small piece of driftwood about 7 feet from the water line. In November, 1968, large numbers of G. oregonensis were actively crawling out of the water on to the sides and tops of large partially submerged logs and were comingling with active Porcellio scaber during a light rain at Albert Head Lagoon; fewer animals were seen exposed to air after the rain had stopped. In the summer of 1968, an animal was observed exposed to air on top of a floating clot of Enteromorpha sp. when the water temperature was about 30°C. Continuing laboratory experiments suggest that G. oregonensis emerges from water far more frequently at high water temperatures than at low water temperatures.

In Albert Head Lagoon, G. oregonensis has been found in salinities lower than 2 °/oo and in water temperatures from about 5°C to 31°C. Relative humidities adjacent to animals under moist logs averaged 97% RH at 11°C to 16°C in May, 1969. In one case the air surrounding animals was as low as 90% RH at 18°C under a log two feet from water. Some of the animals were conglobated. The ambient relative humidity ten feet

above the water averaged 87% at 14°C. Long term meteorological mean

relative humidites vary from 76% in May to 88% in December in nearby

Victoria (Canada Department of Transport. Meteorological Branch, 1967).

At Tomales Bay conglobating individuals of G. oregonensis were

observed under rocks up to five feet from water. The humidities adjacent

to the dry upper surfaces of the rocks were about 82% RH, but humidities

beneath the rocks averaged 93% RH at 19°C to 22°C. The cuticles of

some of the animals were dull and somewhat desiccated. In short,

G. oregonensis occasionally emerge from water into air and are often

stranded in air by tides. When these situations occur, animals usually

frequent moist microhabitats that probably seldom fall below about

90% RH.

## Laboratory Experiments

### Response Intensity

Table 1 presents the mean numbers of position recordings between

one side of the chambers and the other side within each condition.

The two sides are not significantly different from each other in control

animals in constant 98% and 76% RH. This result suggests that no

extraneous environmental factors cloaked the effects of humidity on the

orientation behavior of experimental animals. In contrast animals in

humidity gradients were recorded significantly more often in the humid

side than in the dry side. Therefore, G. oregonensis responds positively

to high humidity conditions. Figures 2 and 3 graphically portray these

data as mean position frequencies at each chamber position. In figure 2

the mean position frequencies of the two gradient conditions vary

directly with relative humidity and inversely with saturation deficiency.

Table 1. Mean numbers of positions in each side (humid and dry sides of gradients) within each condition. $N_A$ is the number of animals tested. $N_P$ is the number of positions recorded. * designates statistical significance at the 95% probability level; † is <u>not</u> significant.

| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | $N_P$ | Numbers of Positions | | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|
| | | | | $\bar{X}$ Side 1 (Humid) | $\bar{X}$ Side 2 (Dry) | | |
| Gradient | 100 | 34 | 2006 | 56.38 | 3.62 | 44.65* | <.005 |
| Gradient | 25 | 30 | 1770 | 53.83 | 6.17 | 36.29* | <.005 |
| 98% RH | 25 | 25 | 1475 | 31.68 | 28.32 | .093† | >.50 |
| 76% RH | 25 | 21 | 1239 | 31.57 | 28.43 | .076† | >.50 |

Figure 3.  Mean position frequencies at each chamber position of 25%
SW acclimated control animals at 98% and at 76% relative
humidity (RH).  Theoretical thigmo-response positions are
also shown.
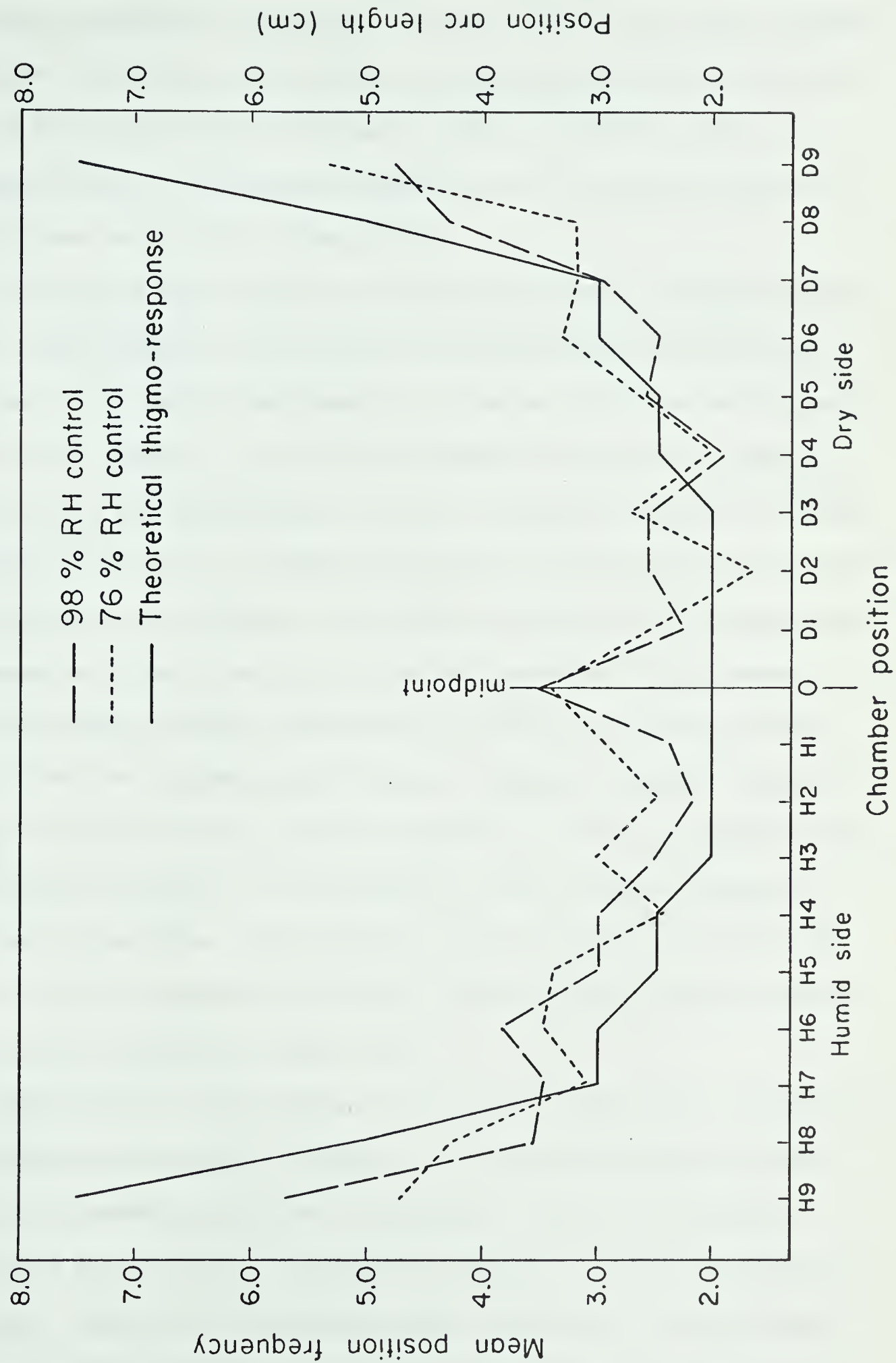
G. oregonensis was recorded more frequently at the highest humidity (or lowest saturation deficiency) available than at any lower humidity condition. Saturation deficiency, an estimate of the drying power of air, is the amount by which the water vapor in air falls short of saturation and is a convenient expression of the combined effects of humidity and temperature (Edney, 1957).

In figure 3 both controls correspond well with a U-shaped theoretical thigmo-response curve which would approximately describe the positions of an animal that moved at a constant speed around the perimeter of the chamber. In fact many animals in the control chambers, as well as in the experimental chambers, displayed conspicuous thigmo-responses; one side of a moving animal was often adjoined to or adjacent to the sides of the chambers. The paths of animals with strong thigmo-responses were nearly circular around the periphery of the chamber. Although thigmo-responses characterized nearly all animals to some extent, the low frequency oscillations in parts of appendix figures 9, 10, and 12 most obviously show this behavior. Thigmo-responses in the laboratory are related to the rugophilic and crevicolous behavior observed in the field. The frequency peaks at positions H6 and 0 in figure 3 and at position H6 in figure 2 are probably recording errors due to poor visibility in these areas.

Comparisons of mean position proportions between the conditions in the four groups are shown in Table 2. Comparing 100% SW acclimated animals in gradients with 25% SW acclimated animals in gradients revealed a statistically significant difference in mean position proportions. The 100% SW acclimated animals demonstrate a more intense response to high humidities than the 25% SW acclimated animals. This

Table 2. Mean position proportions between humidity-salinity
conditions. A mean position proportion is the number
of position records on the humid side divided by the number
of position records on both sides. (Symbols as in Table 1.)

| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | Position Proportions | | |
|---|---|---|---|---|---|
| | | | $\bar{X}$ | F | P |
| Gradient | 100 | 34 | .94 | 6.89* | <.025 |
| Gradient | 25 | 30 | .90 | | |
| Gradient | 25 | 30 | .90 | 183.98* | <.001 |
| 98% RH | 25 | 25 | .53 | | |
| Gradient | 25 | 30 | .90 | 162.19* | <.001 |
| 76% RH | 25 | 21 | .53 | | |
| 98% RH | 25 | 25 | .53 | .0061† | >.75 |
| 76% RH | 25 | 21 | .53 | | |

difference in behavior between the two groups suggests that previous ambient salinity histories may affect the humidity behavior of air-exposed animals through intermediate physiological changes. This fairly subtle difference in the distribution of the two groups in the chambers was not apparent during the experiments.

Differences in mean position proportions between the 25% SW acclimated gradient animals and the similarly acclimated high and low humidity controls were significantly different (Table 2). Gradient animals spent a disproportionate amount of time on the humid side of the chamber while animals in the two constant humidities were recorded about the same number of times on each side. The total mean excess percentages, a similar measure of response intensity, also shows these differences (Table 3). Not surprisingly, the two controls are not significantly different from each other (Table 2). To examine the variability within each condition, position patterns of animals with high response intensities (Appendix figures 1, 4, 7, and 10), low response intensities (Appendix figures 3, 6, 9, and 12), and more typical response intensities (Appendix figures 2, 5, 8, and 11) are illustrated here.

Table 3 also exposes the results of significance tests of the mean position proportions during each half of the 30 minute experimental period for each condition. Mean excess percentages for each condition in each half are also shown. In the animals tested in gradients, response intensities towards the humid side are significantly higher in the second half of the experimental periods than in the first half. Desiccation during the course of the experiments may increase response intensities. Response intensities between the two halves of the experimental period were not significantly different in either control condition.

Table 3. Mean excess percentages and mean position proportions for the 30 minute experimental period (total) and for each half (15 minutes) of the experimental period within each condition.

Mean excess percentage = $\dfrac{\text{number humid side positions} - \text{number dry side positions}}{\text{total number positions}} \times 100$

(Symbols as in Table 1.)

| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | Mean Excess Percentages | | | Mean Position Proportions | | | F | P |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total | First 15 min | Second 15 min | Total | First 15 min | Second 15 min | | |
| Gradient | 100 | 34 | +87.94 | +84.8 | +91.0 | .9397 | .9240 | .9549 | 4.88* | <.05 |
| Gradient | 25 | 30 | +79.44 | +74.1 | +84.8 | .8972 | .8706 | .9239 | 6.35* | <.025 |
| 98% RH | 25 | 25 | +5.59 | +0.66 | +10.5 | .5280 | .5033 | .5527 | 2.04† | >.10 |
| 76% RH | 25 | 21 | +5.23 | +14.3 | -3.8 | .5262 | .5714 | .4809 | 3.55† | >.05 |

## Activity, Speed, and Stops

Mean minimum activities and mean minimum speeds of the four groups are shown in Table 4. In all cases mean minimum speeds are higher than mean minimum activities since stops are not considered in the former calculation. The same trends are apparent in both the activity and the speed data. The 100% SW and 25% SW acclimated animals in the gradient conditions exhibit significantly different mean minimum activities and speeds. This difference again lends support to the hypothesis that humidity behavior may be affected by previous salinity conditions. In the two gradient conditions, mean minimum activities in the first half and second half of the experimental period were not significantly different (Table 5).

As in the position proportion data, the mean minimum speeds and activities of 25% SW acclimated animals tested in gradients are significantly different from both control conditions. The gradient animals were slower than the control animals. Again, however, no significant difference occurred between the two humidity control conditions. This last result seems contradictory if G. oregonensis, like oniscoids, displays a negative hygro-ortho-kinesis. The animals tested in a constant 76% RH might be expected to move significantly faster than the 100% RH animals. Another way of approaching this orthokinesis problem is to compare the mean minimum activities in the humid and dry sides of the chamber within each gradient condition (Table 6). This calculation was accomplished on those animals that were recorded at least twice in the dry side in the 30 minute experimental period. Again, the mean activities on the two sides were not significantly different from each other in either of the gradient conditions. However, the sample sizes

Table 4. Mean minimum activities and mean minimum speeds between
humidity-salinity conditions. (Symbols as in Table 1.)

| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | Mean Minimum Activities (cm/min) | | | Mean Minimum Speeds (cm/min) | | |
|---|---|---|---|---|---|---|---|---|
| | | | $\bar{X}$ | F | P | $\bar{X}$ | F | P |
| Gradient | 100 | 34 | 9.37 | 5.40* | <.025 | 11.50 | 11.89* | <.005 |
| Gradient | 25 | 30 | 11.34 | | | 14.04 | | |
| Gradient | 25 | 30 | 11.34 | 27.61* | <.001 | 14.04 | 17.56* | <.001 |
| 98% RH | 25 | 25 | 16.25 | | | 17.33 | | |
| Gradient | 25 | 30 | 11.34 | 14.90* | <.001 | 14.04 | 4.46* | <.05 |
| 76% RH | 25 | 21 | 15.61 | | | 16.23 | | |
| 98% RH | 25 | 25 | 16.25 | .239† | >.50 | 17.33 | .869† | >.25 |
| 76% RH | 25 | 21 | 15.61 | | | 16.23 | | |

Table 5. Mean minimum activities for the 30 minute experimental
period (total) and for each half (15 minutes) of the
experimental period within each gradient condition.
(Symbols as in Table 1.)

| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | Mean Minimum Activities (cm/min) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Total $\bar{X}$ | First $\bar{X}$ 15 min | Second $\bar{X}$ 15 min | F | P |
| Gradient | 100 | 34 | 9.37 | 9.07 | 9.59 | 0.293† | >.75 |
| Gradient | 25 | 30 | 11.34 | 11.99 | 10.67 | 2.05† | >.10 |

Table 6. Mean minimum activities of animals in humid and dry sides within each gradient condition. Only animals that were recorded at least twice in the dry side were included. (Symbols as in Table 1.)

| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | Mean Minimum Activities (cm/min) | | | | F | P |
|---|---|---|---|---|---|---|---|---|
| | | | Humid Side | | Dry Side | | | |
| | | | $N_P$ | $\bar{X}$ | $N_P$ | $\bar{X}$ | | |
| Gradient | 100 | 21 | 1181 | 9.91 | 58 | 11.23 | 0.87† | >.25 |
| Gradient | 25 | 18 | 982 | 11.64 | 80 | 9.84 | 1.81† | >.10 |

for the dry side were small (about 5% of the total number of distance records for the 100% SW acclimated animals and about 8% of the total for the 25% SW acclimated animals). Variation in these small samples may account for the low mean minimum activity on the dry side (9.84 cm/min) in the 25% SW acclimated animals (Table 6). Animals with typical speeds for their respective conditions include those shown in appendix figures 2 to 6. Appendix figure 7 portrays the positions of an unusually fast animal, and appendix figures 9 and 10 show slow animals.

Comparisons of mean stop times and total numbers of stops between the conditions of each group are shown in Table 7. The total mean stop times and the mean total numbers of stops between the 100% SW and the 25% SW acclimated gradient animals were not significantly different. However, if segregated into humid side and dry side mean stop times (Table 8), the 25% SW acclimated animals and the 100% SW acclimated animals in gradients are significantly different from each other in both sides of the chamber. Animals acclimated to 100% SW stop longer in the humid side than do animals acclimated to 25% SW and _vice_ _versa_ in the dry side. In contrast total numbers of stops between the gradient conditions are not significantly different in either side when stops were corrected to equal time periods for each side (Table 9). These naked stop data, then, provide equivocal evidence for the hypothesis of an osmotic or ionic mechanism of humidity reception, but stop data are also part of the information in the significant activity differences alluded to previously.

Significant differences also surfaced when both controls were tested with the 25% SW acclimated gradient animals (Table 7). The control animals exhibit shorter stops and fewer stops than the animals

Table 7. Mean stop times and total numbers of stops between humidity-
salinity conditions. The symbol $t_s$ designates a Wilcoxon
two-sample test of significance. (Other symbols as in Table 1.)

| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | Mean Stop Times (secs) | | | Total Numbers of Stops | | |
|---|---|---|---|---|---|---|---|---|
| | | | $\bar{X}$ | F | P | $\bar{X}$ | $t_s$ | P |
| Gradient | 100 | 34 | 18.67 | 1.48† | >.05 | 19.38 | .815† | >.40 |
| Gradient | 25 | 30 | 16.46 | | | 21.50 | | |
| Gradient | 25 | 30 | 16.46 | 6.65* | <.025 | 21.50 | 3.97* | <.001 |
| 98% RH | 25 | 25 | 11.42 | | | 10.16 | | |
| Gradient | 25 | 30 | 16.46 | 9.52* | <.005 | 21.50 | 9.09* | <.001 |
| 76% RH | 25 | 21 | 10.37 | | | 7.52 | | |
| 98% RH | 25 | 25 | 11.42 | .206† | >.50 | 10.16 | 1.12† | >.20 |
| 76% RH | 25 | 21 | 10.37 | | | 7.52 | | |

Table 8. Mean stop times of animals in humid and dry sides between
100% SW and 25% SW acclimated gradient tested animals.
Only animals that were recorded at least twice in the dry
side were included. (Symbols as in Table 1.)

| | | | Mean Stop Times (sec) | | | | | |
| | | | Humid Side | | | Dry Side | | |
| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | $\bar{X}$ | F | P | $\bar{X}$ | F | P |
|---|---|---|---|---|---|---|---|---|
| Gradient | 100 | 21 | 19.44 | 2.59* | <.05 | 2.40 | 2.85* | <.05 |
| Gradient | 25 | 18 | 15.79 | | | 5.52 | | |

Table 9.  Total numbers of stops in humid  and dry sides between 100%
          SW and 25% SW acclimated gradient tested animals.  Only animals
          that were recorded at least twice in the dry side were included.
          Numbers of stops were multiplied by correction factors to
          adjust for the unequal time periods spent in each side.  The
          symbol $U_s$ designates a Wilcoxon two-sample test of significance.
          (Other symbols as in Tables 1 and 7.)

| Humidity Conditions | Acclimation Salinities (% sea water) | Total Numbers of Stops | | | | | | | |
| | | Humid Side | | | | Dry Side | | | |
| | | $N_A$ | $\bar{X}$ | $t_s$ | P | $N_A$ | $\bar{X}$ | $U_s$ | P |
| Gradient | 100 | 21 | 9.83 | 1.54† | >.10 | 6 | 6.65 | 28.0† | >.10 |
| Gradient | 25 | 18 | 13.06 | | | 9 | 5.65 | | |

in gradients. Mean stop times and total numbers of stops between the two controls were not significantly different, however. As with speed and activity differences, this result is not characteristic of orthokinetic responses. Comparing the humid and dry sides within each gradient condition again, the longer stops in the humid side were significantly different from the shorter stops in the dry side in both conditions (Table 10). Moreover, subsequent to a correction for equal time periods for each side, significantly more stops were recorded in the humid side than in the dry side in the 25% SW acclimated animals; no such difference appeared in the numbers of stops between the two sides in the 100% SW acclimated animals. In summary, the stop data supports the orthokinesis problem equivocally.

## Turns

Table 11 presents the total numbers of turns, the numbers of ordinary turns, and the numbers of reversal turns of the four groups of humidity-salinity conditions. Mean numbers of ordinary, reversal, and total turns were somewhat greater in the 25% SW acclimated gradient condition than in the 100% SW gradient condition. However, differences were not significant. Turn data, then, does not support the hypothesis of an osmotic or ionic mechanism of humidity reception. Comparisons of the 25% SW acclimated gradient condition with both controls revealed significantly fewer reversal turns in the control animals than in the gradient animals. These differences also appeared in the total number of turns category. These data suggest an orientation response similar to a positive hygro-klino-taxis. Appendix figure 1 portrays a response pattern with many reversal turns; appendix figures 2 and 4 show more typical numbers of reversal turns, and response patterns with few or no

Table 10. Mean stop times and total numbers of stops in humid and dry sides within each gradient condition. Numbers of stops were corrected as in Table 9. (Symbols as in Tables 1, 7, and 9.)

| Humidity Conditions | Acclimation Salinities (% sea water) | Mean Stop Times (sec) | | | | | Total Numbers of Stops | | | | | |
| | | $N_A$ | Humid Side | Dry Side | F | P | Humid Side | | Dry Side | | $t_s$ & $U_s$ | P |
| | | | | | | | $N_A$ | $\bar{X}$ | $N_A$ | $\bar{X}$ | | |
| Gradient | 100 | 21 | 19.44 | 2.40 | 73.28* | <.001 | 21 | 9.83 | 6 | 6.65 | .993† | >.20 |
| Gradient | 25 | 18 | 15.79 | 5.52 | 23.55* | <.001 | 18 | 13.06 | 9 | 5.65 | 147.0 * | <.001 |

Table 11. Total numbers of turns, numbers of ordinary turns, and numbers of reversal turns between humidity-salinity conditions. (Symbols as in Tables 1 and 7.)

| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | Total Numbers of Turns | | | Numbers of Ordinary Turns | | | Numbers of Reversal Turns | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\bar{X}$ | $t_s$ | P | $\bar{X}$ | $t_s$ | P | $\bar{X}$ | $t_s$ | P |
| Gradient | 100 | 34 | 25.15 | 1.20† | >.20 | 9.91 | 1.23† | >.20 | 15.23 | 5.12† | >.50 |
| Gradient | 25 | 30 | 29.13 | | | 12.70 | | | 16.43 | | |
| Gradient | 25 | 30 | 29.13 | 2.88* | <.01 | 12.70 | 1.74† | >.05 | 16.43 | 6.21* | <.001 |
| 98% RH | 25 | 25 | 18.28 | | | 16.64 | | | 1.64 | | |
| Gradient | 25 | 30 | 29.13 | 6.59* | <.001 | 12.70 | 1.80† | >.05 | 16.43 | 10.33* | <.001 |
| 76% RH | 25 | 21 | 19.95 | | | 18.14 | | | 1.81 | | |
| 98% RH | 25 | 25 | 18.28 | .320† | >.50 | 16.64 | 0.31† | >.50 | 1.64 | 0.334† | >.90 |
| 76% RH | 25 | 21 | 19.95 | | | 18.14 | | | 1.81 | | |

31

reversal turns are figured in appendix figures 3, 6, 7, 8, 10, 11, and 12. Although mean numbers of ordinary turns were greater in the control animals than in the animals in gradients, the differences were not statistically significant. Finally, comparisons between the two control conditions were not significantly different in any of the turn categories; in all categories the low humidity control means exceeded the high humidity control means.

To examine the questions posed by the control conditions further, humid and dry sides were compared in each of the turn categories within both gradient conditions (Table 12). The mean numbers of turns on both humid and dry sides were corrected to equal time periods for each side. In both conditions mean numbers of ordinary turns were greater, though not significantly, in the dry side than in the humid side. From this data the existence of a hygro-klino-kinesis in G. oregonensis seems unlikely. There were significantly fewer reversal turns in the dry side than in the humid side in the 25% SW acclimated animals in gradients, but not in the 100% SW acclimated animals in gradients. As with the reversal turns, there was a significant difference between the two sides in total numbers of turns in the 25% SW acclimated animals, but not in the 100% SW acclimated animals.

Distances between total turns in the dry and in the humid sides in both the 100% SW and 25% SW acclimated gradient conditions are shown in Table 13. There are no significant differences between the two gradient conditions in either the humid or the dry sides. This result reiterates that turn data does not relate to the hypothesis of an osmotic or ionic mechanism of humidity reception. However, after a time period correction, distances between turns were significantly greater in the humid

Table 12. Total numbers of turns, numbers of ordinary turns, and numbers of reversal turns in humid and dry sides within each gradient condition. Only animals that were recorded at least twice in the dry side were included. All classes of turns were corrected as in Table 9. (Symbols as in Tables 1, 7, and 9.)

| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | Total Numbers of Turns | | | | Numbers of Ordinary Turns | | | | Numbers of Reversal Turns | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Humid Side | Dry Side | $t_s$ & $U_s$ | P | Humid Side | Dry Side | $t_s$ & $U_s$ | P | Humid Side | Dry Side | $t_s$ & $U_s$ | P |
| Gradient | 100 | 21 | 13.45 | 11.72 | 1.284† | >.10 | 5.51 | 7.56 | 1.46† | >.10 | 7.93 | 14.17 | 1.167† | >.20 |
| Gradient | 25 | 18 | 14.37 | 10.27 | 178.0* | <.025 | 6.34 | 8.41 | 118.0† | >.10 | 8.03 | 5.70 | 114.5* | <.05 |

Table 13. Distances between total turns in humid and dry sides between 100% SW and 25% SW acclimated gradient tested animals. Only animals that were recorded at least twice in the dry side were included. Distances between turns were corrected as in Table 9. (Symbols as in Tables 1, 7, and 9.)

| Humidity Conditions | Acclimation Salinities (% sea water) | Distances Between Total Turns (cm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Humid Side | | | | Dry Side | | | |
| | | N | $\bar{X}$ | $t_s$ | P | N | $\bar{X}$ | $U_s$ | P |
| Gradient | 100 | 21 | 12.4 | 1.719† | >.05 | 15 | 12.5 | 113.5† | >.10 |
| Gradient | 25 | 18 | 15.3 | | | 14 | 10.8 | | |

side than in the dry side in the 25% SW acclimated animals in gradients (Table 14). Distances between turns, however, were not significantly different between the two sides of the gradient in animals acclimated to 100% SW.

In the two gradient conditions, mean numbers of reversal turns in the first half and the second half of the experimental period were not significantly different (Table 15).

## Evaporative Water Loss

To aid in interpreting the behavior data for the hypothesis of an osmotic or ionic mechanism of humidity reception, an evaporative water loss experiment was run. Mean initial weights, final weights, weight losses, and percent weight losses of 100% SW and 25% SW acclimated animals are shown in Table 16. Although initial weights of the two groups were not significantly different, the percent weight loss between the groups was significantly different. Animals acclimated to 100% SW evaporated only about 3/4 as much water as animals acclimated to 25% SW in the conditions specified in the methods section. If nearly all of the evaporative water loss was from the hemolymph and if the volume of the hemolymph is about 25% of the initial weight (Riegel, 1959a), then the 100% SW acclimated animals lost about 48% of their hemolymph volume and increased their hemolymph concentration from about 70% SW to about 104% SW. Similarly, the 25% SW acclimated animals lost about 64% of their hemolymph volume and increased their hemolymph concentration from about 28% SW to about 46% SW. No animals died during the one hour time period of the experiment. Most of the animals were partially conglobated throughout the experiment.

Table 14. Distances between total turns in humid and dry sides within each gradient condition. Only animals that were recorded at least twice in the dry side were included. Distances between turns were corrected as in Table 9. (Symbols as in Tables 1, 7, and 9.)

| | | Distances Between Total Turns (cm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Humid Side | | Dry Side | | | |
| Humidity Conditions | Acclimation Salinities (% sea water) | N | $\bar{X}$ | N | $\bar{X}$ | $t_s$ & $U_s$ | P |
| Gradient | 100 | 21 | 12.4 | 15 | 12.5 | 0.594† | >.50 |
| Gradient | 25 | 18 | 15.3 | 14 | 10.8 | 170.5 * | <.05 |

Table 15.  Mean numbers of reversal turns for the 30 minute experimental
           period (total) and for each half (15 minutes) of the experi-
           mental period within each gradient condition.  (Symbols as
           in Tables 7 and 9.)

| Humidity Conditions | Acclimation Salinities (% sea water) | $N_A$ | Mean Numbers of Reversal Turns | | | | |
|---|---|---|---|---|---|---|---|
| | | | Total | First 15 min | Second 15 min | $U_s$ & $t_s$ | P |
| Gradient | 100 | 34 | 15.2 | 7.3 | 7.9 | 650.5† | >.4 |
| Gradient | 25 | 30 | 16.4 | 8.8 | 7.6 | 0.180† | >.9 |

Table 16.  Mean initial weights, final weights, weight losses, and
percent weight losses of 100% SW and 25% SW acclimated
animals in an evaporative water loss experiment.  (Symbols
as in Table 1.)

| Acclimation Salinities (% sea water) | $N_A$ | Initial Weight | | | $\bar{X}$ Final Weight (mg) | $\bar{X}$ Weight Loss (mg) | Percent Weight Loss | | |
|---|---|---|---|---|---|---|---|---|---|
| | | $\bar{X}$ (mg) | F | P | | | $\bar{X}$ | F | P |
| 100 | 36 | 13.801 | 1.625† | >0.10 | 12.186 | 1.615 | 11.71 | 29.30* | <.001 |
| 25 | 36 | 12.927 | | | 10.917 | 2.010 | 15.66 | | |

DISCUSSION

## Behavior

Many intertidal animals along the northwest coast of North America are potentially, at least, exposed to high temperature and to low humidity stresses during the summer and to low temperature and to low salinity stresses during the winter because the mixed, semidiurnal tides are low in the daytime and high at night in summer and _vice versa_ in winter. Rainfall is higher in the winter than in the summer. These severe conditions are meliorated in part by mechanisms of behavioral regulation in many intertidal animals. G. oregonensis is no exception. Preliminary unpublished data (my own) suggest that G. oregonensis evaporates water fairly rapidly, especially in the first hour of exposure, and tolerates humidity conditions below about 90% RH in the order of hours, particularly at high air temperatures. Because of this humidity tolerance below saturation and because G. oregonensis ordinarily frequents very high humidity microhabitats when exposed to air, movements away from high humidity microhabitats are probably prevented by orientation responses.

Generally, isopods and amphipods that orient to humidity conditions, including G. oregonensis, respond positively towards high humidities, usually for the highest humidity available. However, for some species response intensities may not be highest or activities may not be lowest at the highest humidity available when animals are not sufficiently desiccated (Cloudsley-Thompson, 1952, 1956; Warburg, 1968), at high and low temperatures (Cloudsley-Thompson, 1956; Warburg, 1964), in high or low humidity ranges (Miller, 1938; Waloff, 1941; Warburg, 1964), under

certain light conditions (Cloudsley-Thompson, 1952; Warburg, 1964), in steep gradients (Williamson, 1951), or in certain combinations of these conditions (Warburg, 1964). Most of these exceptions have been demonstrated in mesic- and especially xeric-adapted oniscoids.

In the terminology of Kennedy (1945), a negative hygro-ortho-kinesis of speed, activity, or both has been demonstrated in several oniscoid isopods and several talitrid and gammarid amphipods. The importance of an activity orthokinesis in the orientation of _Porcellio scaber_ (Gunn, 1937) and in three talitrids (Williamson, 1951) in humidity gradients has been elucidated; Perttunen (1961) considered a negative hydro-ortho-kinesis of both speed and activity to be the dominant responses in _Ligia italica_. Waloff (1941) also described both speed and activity orthokineses in _Oniscus asellus_ and _Porcellio scaber_, but her _Armadillidium vulgare_ may exhibit only a speed orthokinesis. Lagerspetz (1963) analyzed a significant activity negative orthokinesis in the two gammarids, _Gammarus duebeni_ and _G. oceanicus_, but Lagerspetz and Lehtonen (1961) failed to show a significant hygro-ortho-kinesis in the asellid, _Asellus aquaticus_.

In this study much of the evidence suggests that _G. oregonensis_ has neither a speed nor an activity hygro-ortho-kinesis. Speeds, activities, stop times, and numbers of stops were not significantly different between the high and low humidity controls. Changing interactions between humidity conditions and water balance, that are more pronounced in gradient conditions than in constant conditions, may provide important feedback information for regulating behavioral responses. Such feedback may be diminished in constant conditions. In addition mean activities in the humid and dry sides of the gradient conditions were not

significantly different from each other, and there was no difference

in numbers of stops between the two sides in the 100% SW acclimated

animals.  In contrast, significantly more stops were recorded in the

humid side than in the dry side from the 25% SW acclimated animals, and

both 100% SW acclimated and 25% SW acclimated animals exhibited longer

stops on the humid side than on the dry side.  Longer stops in higher

humidity conditions probably account for the peak in the mean position

frequency curve at the highest humidity available (Fig. 2).  G. oregonen-

sis may exhibit a negative hygro-ortho-kinesis that is easily masked

by other orientation reactions and that functions mainly in shallow

humidity gradient situations, but such a reaction was not demonstrated

convincingly in the steep gradient conditions used in this study.  Simi-

larly, Lagerspetz and Lehtonen (1961) suggested that non-significant

kineses were especially evident in those Asellus failing to show klino-

tactic behavior, and Williamson (1951) states that orthokineses are

most important in weak hygro-reactions.

Although the existence of a negative hygro-ortho-kinesis in G.

oregonensis is still questionable, another reaction dominated the humi-

dity behavior of G. oregonensis in this study.  Significantly more

reversal turns were recorded from the 25% SW acclimated gradient condi-

tion than from the two 25% SW acclimated controls.  Animals in the gra-

dients maintained themselves in high humidity conditions by effecting

numerous reversal turns in the middle areas of the gradients.  Nearly all

reversal turns reversed the direction of an animal that was moving into

dryer humidities.  Most reversal turns were sharp and were abruptly and

rapidly executed, often after a brief stop.  In most of these characteris-

tics, reversal turns were unlike ordinary turns.  The angles of the turns

were about $180° \pm 30°$. After the execution of a reversal turn, animals moved

fairly directly to the highest humidity available. Since there were significantly more reversal turns on the humid side than on the dry side of the midline in 25% SW acclimated animals in gradients and since the humidity at the midline was about 84% to 87% RH, humidites of about 85% to 90% RH at 21°C may trigger reversal turns in G. oregonensis. This humidity range is closely correlated with field data; animals were rarely found in humidities lower than about 90% RH.

The reversal turn reaction of G. oregonensis can be variously interpreted. If the reversal turn reaction orients the animal in line with the stimulus and if locomotion is towards or away from it by successive comparisons of stimulus intensities, the reversal turn reaction might be a positive hygro-klino-taxis. Lagerspetz and Lehtonen (1961) and Perttunen (1961) interpreted 180° turns in Asellus and Ligia, respectively, as hygro-klino-taxes. This reaction dominated all other humidity orientation reactions in Asellus. Secondly, the reversal turn reaction might be a titubant reaction (Ewer and Bursell, 1950) which is a reaction at a boundary by animals which do not have a klino-kinesis with sensory adaptation; this reaction is characterized by slowing or stopping, followed by a tactic reorientation. Finally, if the reversal turn reaction is an undirected change in turning frequency/ unit time dependent on an abrupt change in stimulus intensity, it may be an extreme klinokinesis with rapid sensory adaptation. This extreme klinokinesis was described by Gunn (1937) as a hygrophobotaxis in P. scaber and by Williamson (1951) and Lagerspetz (1963) as part of their klinokinesis in talitrids and gammarids, respectively. Although the reversal turn reaction in G. oregonensis resembles a titubant reaction, classification of the reversal turn reaction will be deferred until more

detailed comparative studies of the characteristics of the various
reactions are accomplished.

In contrast to reversal turns, there were not significantly more
ordinary turns or total turns in the high humidity control than in the
low humidity control, and there were not significantly more ordinary
turns in the humid side of the gradients than in the dry side. Conse-
quently, the presence of a hygro-klino-kinesis similar to Ullyott's
(1936) photophobotaxis in the triclad, Dendrocoelum lacteum, seems
improbable. Nevertheless, most of the previous humidity behavior studies
on oniscoids and amphipods have described the presence of a hygro-
klino-kinesis similar to Ullyott's photophobotaxis (Waloff, 1941;
Williamson, 1951; Perttunen, 1961; and Lagerspetz, 1963). Both Perttunen
(1961) and Lagerspetz (1963) found the number of turns/unit time to
be greater in the dry sides of their gradients than in the humid sides.
However, animals that aggregate in high humidities by a klinokinesis
would be expected to turn more often/unit time in the humid side than
in the dry side as in the studies of Waloff (1941) and Williamson (1951).
Waloff (1941) recognized that the more frequent turning of animals in
her humid conditions than in her dry conditions differed from Ullyott's
observations; there was a kinetic effect but no sensory adaptation in
her isopods. Lindqvist (1968) has argued that klinokinetic and ortho-
kinetic orientation are merely results of different activity levels.

Summarizing, G. oregonensis orients towards high humidities with a
reversal turn reaction and possibly a negative hygro-ortho-kinesis. The
former reaction dominates in steep gradients while the latter reaction
may be more evident in shallow gradients. There was no evidence to
support the existence of klinokinetic orientation to humidity in G.
oregonensis.

Reception

Studies on specific humidity reception in arthropods have been critically reviewed by Syrjämäki (1962). In isopods and amphipods attempts to show the presence of and the localities of specific external hygroreceptors have been accomplished chiefly by removal and masking behavior experiments. Gunn (1937) recorded normal kinetic reactions in Porcellio scaber subsequent to amputation of antennae and uropods and to masking of mouthparts, pseudotracheae, and pleopods. He concluded that receptors probably occur in the thoracic region. In another study, the humidity preferences of Armadillidium vulgare deprived of their antennae were not different from controls, suggesting that reception is not exclusively dependent on receptors in the antennae (Miller, 1938). Furthermore, Janda and Lang (1939) found no changes in humidity preferences in Oniscus murarius and P. scaber after ablation of the antennae or after masking of the head, dorsum, or ventral abdomen. They suggested that the medial and ventral thoracic regions might harbor humidity receptors. Williamson (1951) amputated the antennae of Talitrus saltator and masked the antennules, antennae, and dorsum of Orchestia gammarella and the head, mouthparts, antennules, antennae, dorsum, and thoracic legs of T. saltator in a series of experiments. Again, however, humidity preferences and orientation mechanisms of the experimentals were no different from the controls. Cloudsley-Thompson (1956) also reported that humidity responses in Oniscus asellus were not influenced by ablation of the terminal articles of the antennae or by masking of the mouthparts, antennulae, antennal cones, pleopods, or all of these appendages. Finally, removal of the antennae and antennulae of Asellus aquaticus did not alter reaction intensities, suggesting that humidity

receptors are not located on these appendages (Lagerspetz and Lehtonen, 1961). Unlike the situation in insects, there seems to be no positive evidence for the existence of specific external hygroreceptors in isopods and amphipods from removal and masking experiments. If specific receptors occur in these animals, the receptors are likely to be endo-receptors. However, the presence of external hygroreceptors on the pereipods or the ventral thoracic regions remains a possibility. In a critical evaluation of removal and masking experiments, Lindqvist (1968) has suggested that stress effects on peripheral sensory acuity and possibly on the CNS may confound and invalidate the results of such experiments.

Barring a few exceptions (Warburg, 1964), behavioral responses in oniscoids seem to be more closely correlated with saturation deficiency than with relative humidity (Miller, 1938; Cloudsley-Thompson, 1956). Because definite external reception sites have not been located and because behavioral responses correlate best with saturation deficiency, several workers have suggested that humidity reactions may be influenced by evaporative water loss from the whole body surface (Waloff, 1941; Williamson, 1951; Cloudsley-Thompson, 1956).

Moreover, an evaporimetric mode of reception is implied (Lindqvist, 1968) because only littoral, and not oceanic, amphipods seem to react to humidity differences (Lagerspetz, 1963). Since the ancestors of oniscoid isopods were undoubtedly aquatic forms (Vandel, 1943) and since important selective advantages may have been associated with mechanisms of coping with evaporative water loss in littoral forms, evaporative modes appear to be more parsimonious explanations for the reception of humidity information than hygrometric modes. _G. oregonensis_ evaporates

water in air, and the response intensities of both the 100% SW and the 25% SW acclimated animals increase during the thirty minute experimental period. These data suggest the prevalence of an evaporimetric mode of humidity reception in G. oregonensis as well.

Possible evaporimetric modes of reception could include chemical, osmotic, mechanical, or temperature mechanisms (Pielou, 1940). This study has attempted to test the hypothesis of an internal osmotic or ionic mechanism with a salinity acclimation approach. Gradient tested G. oregonensis acclimated to 100% SW respond to high humidities with a significantly higher response intensity than 25% SW acclimated animals at the same temperature. Animals acclimated to 25% SW did not occur as frequently in the higher humidities as did the 100% SW acclimated animals; the converse was recorded in the lower humidities (Fig. 1). Similar results were obtained by Lagerspetz and Lehtonen (1961) in Asellus aquaticus. In their study animals collected from fresh water pools displayed a mean reaction intensity of +26.7% in a humidity gradient while the intensity of those taken from brackish water of about 6 $^{o}/oo$ was +37.5%. These results suggest that hemolymph concentration may affect humidity behavior. In the present study the 100% SW acclimater animals move significantly slower than the 25% SW acclimated animals, but both groups effected about the same number of reversal turns. These data suggest that activity rate rather than number of reversal turns accounts for the response intensity differences between the 100% SW and the 25% SW acclimated animals. Reversal turns, however, were important in maintaining the overall high response intensities of both of the experimental groups in humidity gradients. Accordingly, mechanisms for the reception of humidity or saturation deficiency

information in G. oregonensis must apply to both activity rates and reversal turns.

When arthropods with relatively permeable cuticles evaporate water and desiccate in air, several attendant consequences may occur more-or-less concurrently: (1) cuticular shrinkage (Bursell, 1955), (2) decreasing hemolymph volumes, (3) increasing concentrations of hemolymph constituents (Widmann, 1936; Parry, 1953; Bursell, 1955; Wilson, 1970), and (4) evaporative cooling (Edney, 1951, 1953). Any one or more of these consequences of evaporation could possibly be instrumental in the reception of humidity or saturation deficiency information. In addition, most of these consequences could peripherally affect locomotory muscle cells or neuromuscular junctions directly, or they could stimulate specialized receptors or neurons.

Little is known of the feasibility of cuticular shrinkage or hemolymph volume mechanisms for the reception of humidity information. After considerable desiccation, asphyxiation and dysfunction of locomotory muscles could result from cuticular shrinkage (Bursell, 1955; Edney and Spencer, 1955) or possibly from decreasing hemolymph volumes and pressures. If asphyxiation was important in the present study, the animals with the highest evaporation rate would move slowest. In G. oregonensis the 25% SW acclimated animals have a higher evaporation rate and move faster than the 100% SW acclimated animals. Consequently, peripheral shrinkage and volume mechanisms are probably not ordinarily important in controlling activity rates in humidity gradients.

In addition to these peripheral asphyxiation mechanisms, mechanoreceptors could be sensitive to cuticular or muscular shrinkage or to decreasing hemolymph volumes or pressures caused by desiccation. After

unsuccessful attempts to demonstrate external pressure receptors in

Oniscus asellus, Friedlander (1964) suggested the possibility of pro-

prioceptors sensitive to muscle or hemolymph pressures.  Also, an

inverse relationship between hemolymph volume and drinking responses,

which is not related to osmotic concentration of the hemolymph, is

known in the blowfly, Phormia regina (Dethier and Evans, 1961), and

these investigators suggest that the effect is probably neurally

mediated via mechanoreceptors sensitive to pressure.  Barton-Browne

(1968) reported the $Cl^-$ and osmotic concentrations of the hemolymph,

in addition to volume effects, to be important variables in the water

ingestion of the blowfly, Lucilia cuprina.  Recent studies demonstrating

an inverse relationship between $Na^+$ uptake and hemolymph volume in

Sphaeroma rugicauda (Harris, 1970) and in Gammarus duebeni (Lockwood,

1970) have reemphasized the probability of hemolymph volume monitoring

mechanisms in crustaceans.  The possibility of desiccation effects on

such mechanisms has not been studied.  Data in the present study cannot

discount the possibility of mechanoreceptors or pressure receptors that

are sensitive to desiccation effects.  However, because such a control

system would sustain interference from molting, feeding, and osmotic

effects over different time courses, some doubt about the efficacy of

such a system seems appropriate.

Desiccation also increases total osmotic concentration of the

hemolymph (Widmann, 1936; Parry, 1953; Bursell, 1955; Wilson, 1970) and

increases hemolymph concentrations of cations (Gross, 1959, 1963).

Different salinities and concentrations of certain cations may affect

activity.  Certain crabs, such as Pachygrapsus crassipes (Gross, 1957),

and the lobster Jasus lalandei (Krijgsman and Krijgsman, 1954) escape

from high and low concentrations of sea water, and Gammarus duebeni

becomes hyperactive when its medium is diluted (Lockwood, 1968).

Furthermore, hemolymph $Mg^{++}$ concentrations are inversely correlated

with activity in 15 species of decapods (Robertson, 1953). High hemo-

lymph concentrations of $Mg^{++}$ may explain the low activity of the land

crab, Gecarcinus lateralis, immediately before and after ecdysis (Bliss,

1962). In electrophysiological studies on a number of decapods, con-

traction amplitudes vary inversely with $Mg^{++}$ concentrations, and concen-

trations of $Mg^{++}$ of 2.5 times normal hemolymph concentration will block

neuromuscular junctions and inhibit contractions (Katz, 1936; Waterman,

1941). Other cations, particularly $K^{+}$ and $Ca^{++}$, may also influence

activity by directly affecting neuromuscular junctions or muscle cells.

In addition, muscle tissue, as well as cuticle and hemolymph, may

dehydrate after relatively mild desiccation. After desiccation of as

little as 9% body weight, both muscle and gill tissue averaged about 30%

less water in Gecarcinus (Gross, 1963). The extent to which such tissue

dehydration is influenced by high ionic concentrations, low tissue

water levels, or both is unknown.

Although the direct effects of high hemolymph concentrations of

cations or of tissue dehydration on locomotory muscles could help

elucidate the low activity rates of the 100% SW acclimated G. oregonensis

after considerable desiccation, such effects probably do not explain

response intensity differences between the 100% SW and 25% SW acclimated

animals early in the experimental period when the absolute amount of

desiccation is minimal (Table 3). Also, all of the direct peripheral

effects are likely to progressively impair contraction and decrease

activity rates during desiccation. However, activity rates and reversal

turns generally do not decrease during the experimental period (Tables

5 and 15), and slow animals occasionally show bursts of activity.

Except for relatively extreme circumstances, it seems unlikely that

peripheral factors can do more than modify activity levels; activity is

probably controlled primarily by the integration of inputs in the CNS

(Kennedy, 1969).

Receptor sensitivity or direct CNS sensitivity to total osmotic

concentration or to specific ions is suggested in a number of behavioral

and electrophysiological studies. Behavioral discrimination of various

salinities and ions has been demonstrated in Crangon vulgaris (Spiegel,

1927); Ligia baudiniana (Barnes, 1935, 1938, 1939, 1940); Asellus

aquaticus, Idotea baltica, and Gammarus spp. (Lagerspetz and Mattila,

1961); Birgus latro (Gross, 1955); and Pachygrapsus crassipes (Gross,

1957). In an electrophysiological study, Krijgsman and Krijgsman (1954)

demonstrated spikes from an abdominal motor nerve of Jasus lalendei that

were correlated with stimulation of the antennulae with concentrated and

diluted sea water. Moreover, in Podophthalamus vigil and Portunus

sanguinolentus, van Weel and Christofferson (1966) have shown that

electroactivity of the antennulae is inversely proportional to stimulus

salinity below about 90% SW, and have suggested that receptors are

located in the flagellum of the antennula. A similar relationship was

noted in Thalamita crenata below about 70% SW (van Weel and Correa,

1966). Similarly, Hughes and Kerkut (1956) and Kerkut and Taylor (1956)

have demonstrated that the impulse frequencies of certain cells in the

pedal ganglia of the slugs Agriolimax reticulatus and Arion ater

are inversely proportional to the osmotic concentration of the perfusion

solutions and that adaptation sometimes occurs subsequent to rapid

changes in the osmotic concentrations in the solutions.

In most of these studies, cells are presumably sensitive to ambient water conditions. However, cells in the slugs (Hughes and Kerkut, 1956; Kerkut and Taylor, 1956) and in the blowfly, Lucilia (Barton-Browne, 1968) are probably sensitive to hemolymph concentrations, and because Birgus can control the concentration of its hemolymph by selecting certain salinities (Gross, 1955), sensitivity to hemolymph concentration probably occurs in this animal as well. The data in the present study suggest that G. oregonensis may also be sensitive to hemolymph concentration. Although the most generalized hemolymph concentration mechanism in G. oregonensis would seem to require only direct CNS or osmoreceptor sensitivity to hemolymph osmotic concentration, sensitivity to certain other hemolymph components remain feasible possibilities. Ligia baudiniana can discriminate between a number of different ions (Barnes, 1940). Furthermore, cation receptors (Hodgson and Roeder, 1956; Evans and Mellon, 1962b), and anion receptors (Steinhardt, 1965 and unpublished data) have been demonstrated electrophysiologically in the labellar sensillae of the blowfly, Phormia regina.

In G. oregonensis activity rates and response intensities are probably affected by hemolymph concentrations. Consequently, the total osmotic concentration of the hemolymph or the concentration of a specific component of the hemolymph may produce changes in receptor cells or neurons exposed to the hemolymph in air-exposed animals that are transpiring water and concentrating their hemolymph. Because evaporation rates (Edney, 1951a; Bursell, 1955), and hemolymph concentrations rates, decrease in isopods during desiccation and because activity rates do not

differ during the experimental period, cells sensitive to hemolymph concentration probably accommodate to changing rates of stimulation.

If receptor activity is inversely proportional to osmotic concentration in G. oregonensis, as in the crab and slug studies, then the 100% SW acclimated animals with high hemolymph concentrations and slow locomotion rates would have a low level of nervous activity, and the 25% SW acclimated animals with low hemolymph concentrations and faster locomotion rates would have a higher level of nervous activity. Similarly, the animals in constant humidities might have very high locomotion rates because their hemolymph concentrations were low and may move faster than the gradient animals with the same low hemolymph concentrations (Table 4) because they turned much less frequently (Table 11). Differences between the high humidity control and the low humidity control may have appeared in a more extended experimental period. The establishment of this relationship will require an electro-physiological approach on the effects of hemolymph concentrations on possible receptors and on the CNS and the consequent changes in motor output and behavior.

Finally, since evaporation rates vary linearly with saturation deficiency over a wide temperature range in most oniscoids (Edney, 1951a), thermoreceptors might detect humidity information via evaporative cooling (Lindqvist, 1968). In Ligia oceanica body temperature depressions up to 7.3°C have been recorded in dry air at 37°C (Edney, 1951b), and cold receptors in insect antennae are sensitive to 0.5°C to 1°C changes in temperature (Lacher, 1964; Loftus, 1966; Schoonhoven, 1967). Winston (1963) has postulated humidity reception by thermo-receptors in the mite, Bryobia praetiosa, from behavioral data. Since

evaporative cooling effects are greatest where surfaces are exposed to air, associated thermoreceptors are likely to be located in or near the cuticle. An evaporative cooling mechanism for the monitoring of humidity information cannot be ruled out in the present study. In fact, because hemolymph concentration effects may not account for immediate reactions (Waloff, 1941), rapid reactions, such as the reversal turns of G. oregonensis, might be effected in response to temperature rate changes exceeding a threshold.

In summary, both hemolymph concentration and evaporative cooling mechanisms may be involved in the reception of humidity information in G. oregonensis. Interactions of more than one mechanism of reception may help to refine behavioral control systems. Verification of the concentration and cooling mechanisms will require further demonstrations of the probability of these mechanisms and of the improbability of other possible mechanisms using a variety of methods and approaches.

## EPILOGUE

Excepting the work on an asellid (Lagerspetz and Lehtonen, 1961) and this study on a sphaeromid, the humidity behavior of only about ten species of isopods in only five families of Oniscoidea has been investigated thus far. Valid quantitative and qualitative comparisons of the humidity behavior between species have been hampered by the different methods, environmental conditions, and interpretations that have been applied in different studies.

Vandel (1943, 1965) has proposed that there were several terrestrial invasions by isopods and that the Oniscoidea are polyphyletic. Our knowledge of the evolution of terrestrialism and of behavior in isopods would be enhanced in the future by exhaustive comparative studies of species in many different oniscoid families (especially those in Vandel's Série tylienne and Série trichoniscienne) and in a number of families of Asellota, Valvifera, and Flabellifera that may be hygroreactive.

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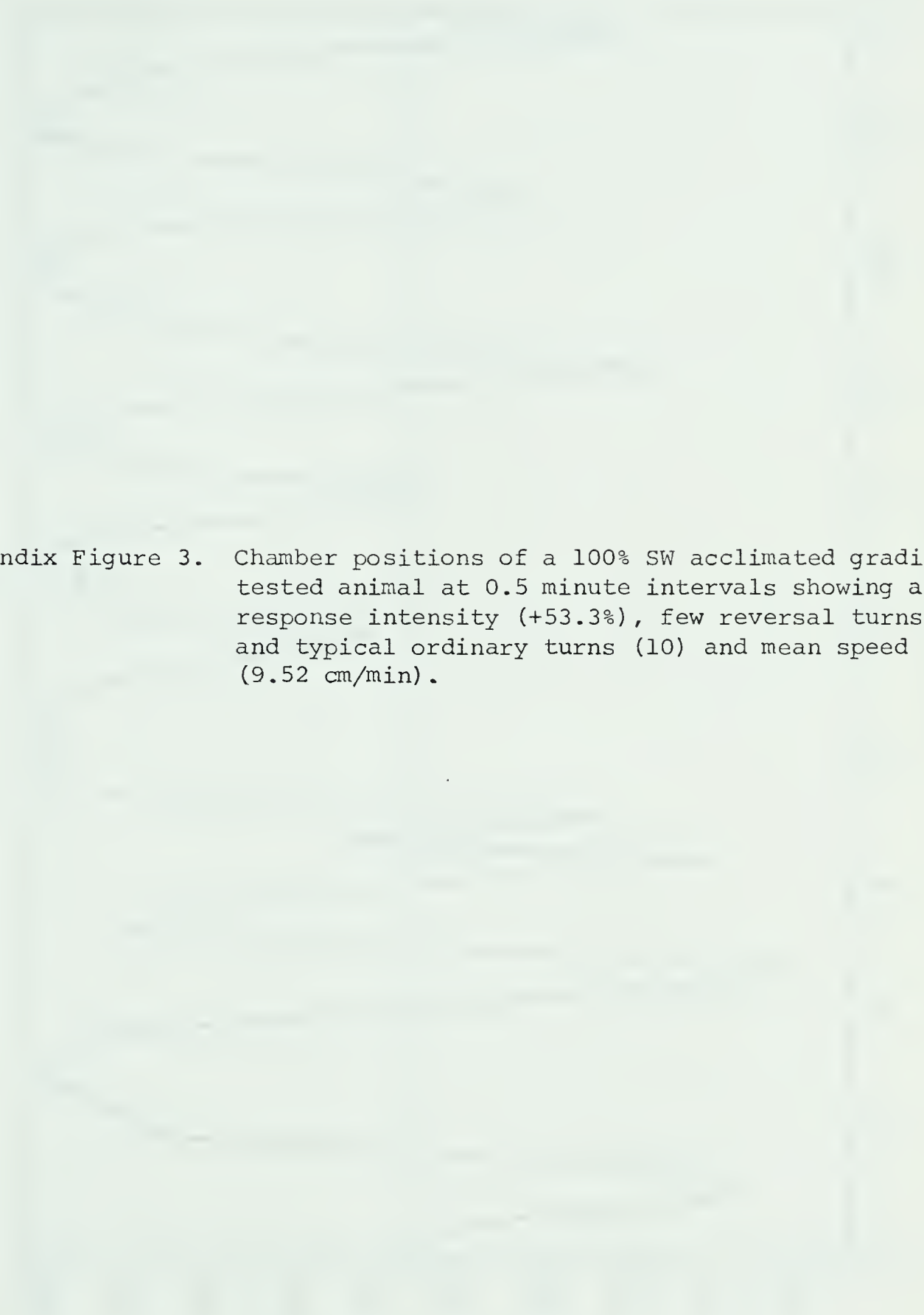
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Appendix Figure 1.   Chamber positions of a 100% SW acclimated gradient
                     tested animal at 0.5 minute intervals showing a
                     high response intensity (+100%), many reversal turns
                     (20), many ordinary turns and a mean speed of 12.17
                     cm/min.  Lines between points in all appendix
                     figures are minimal distances between positions, not
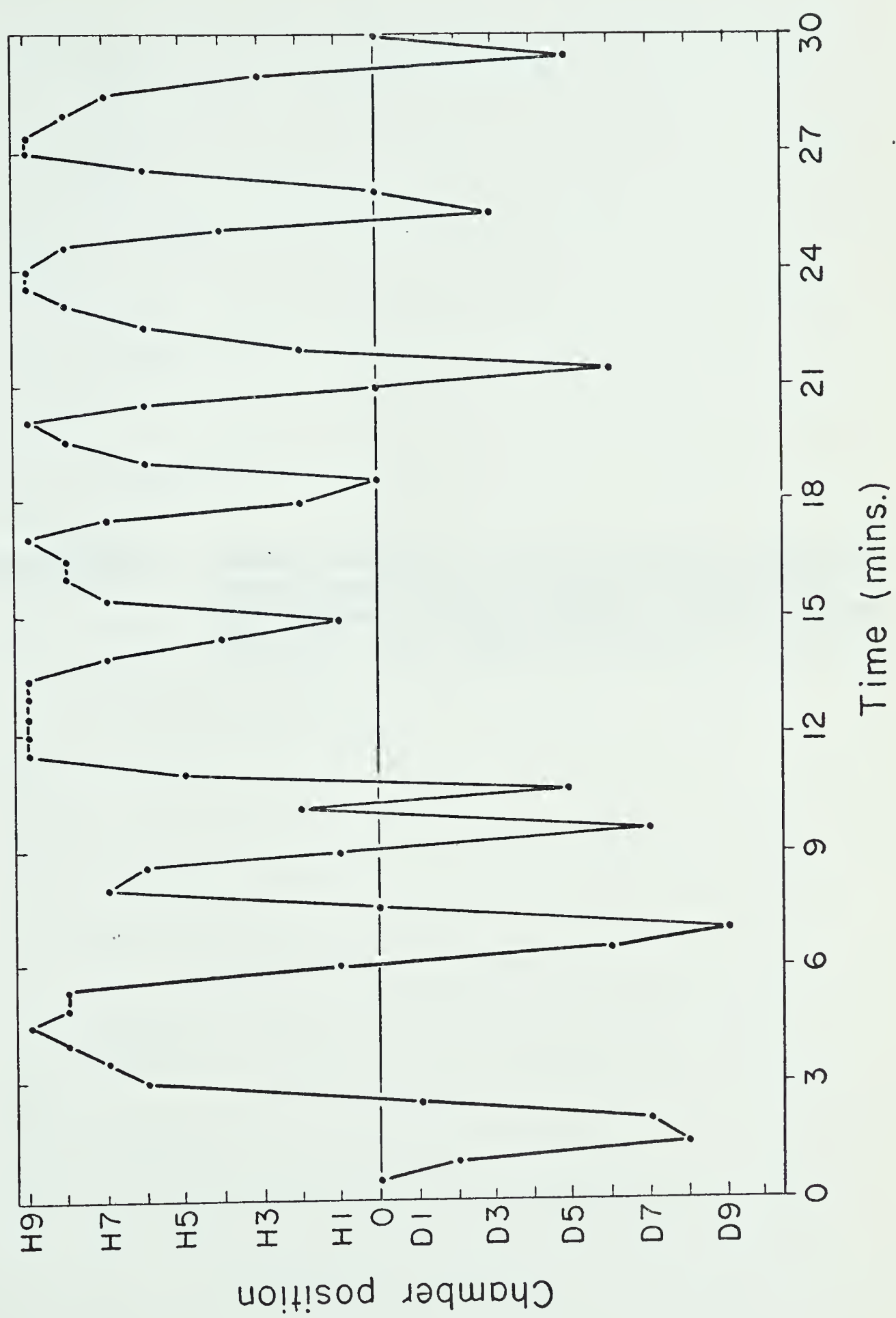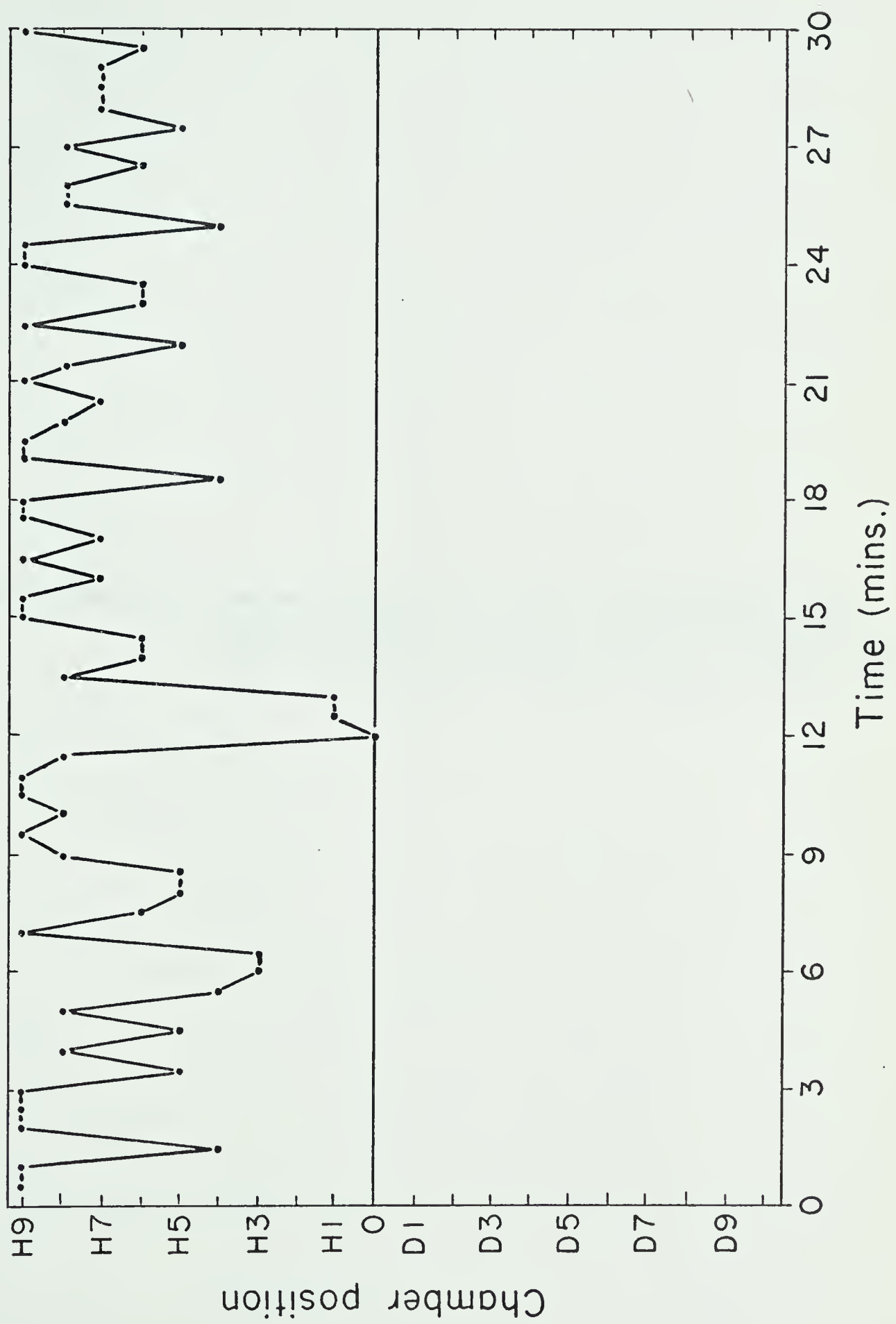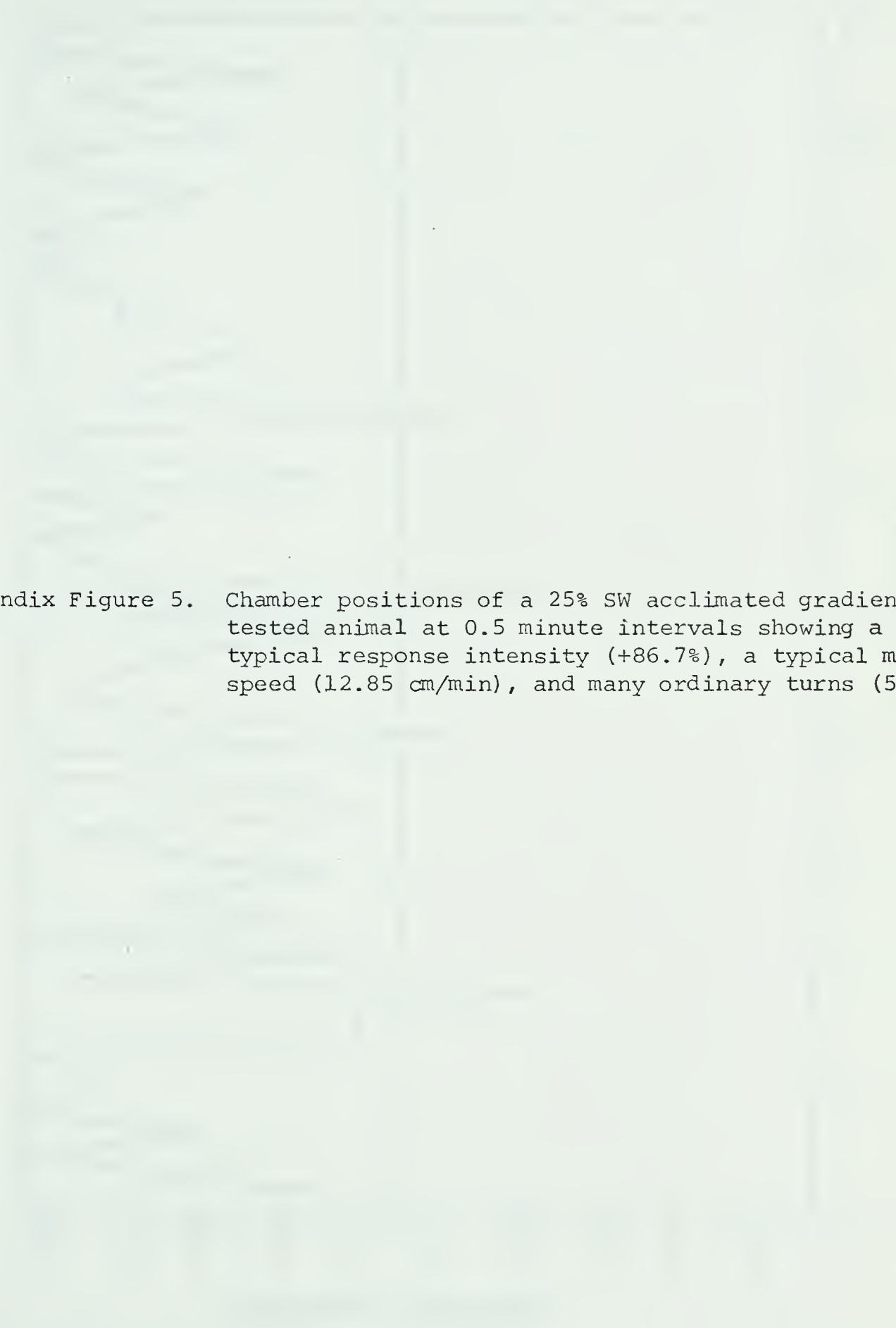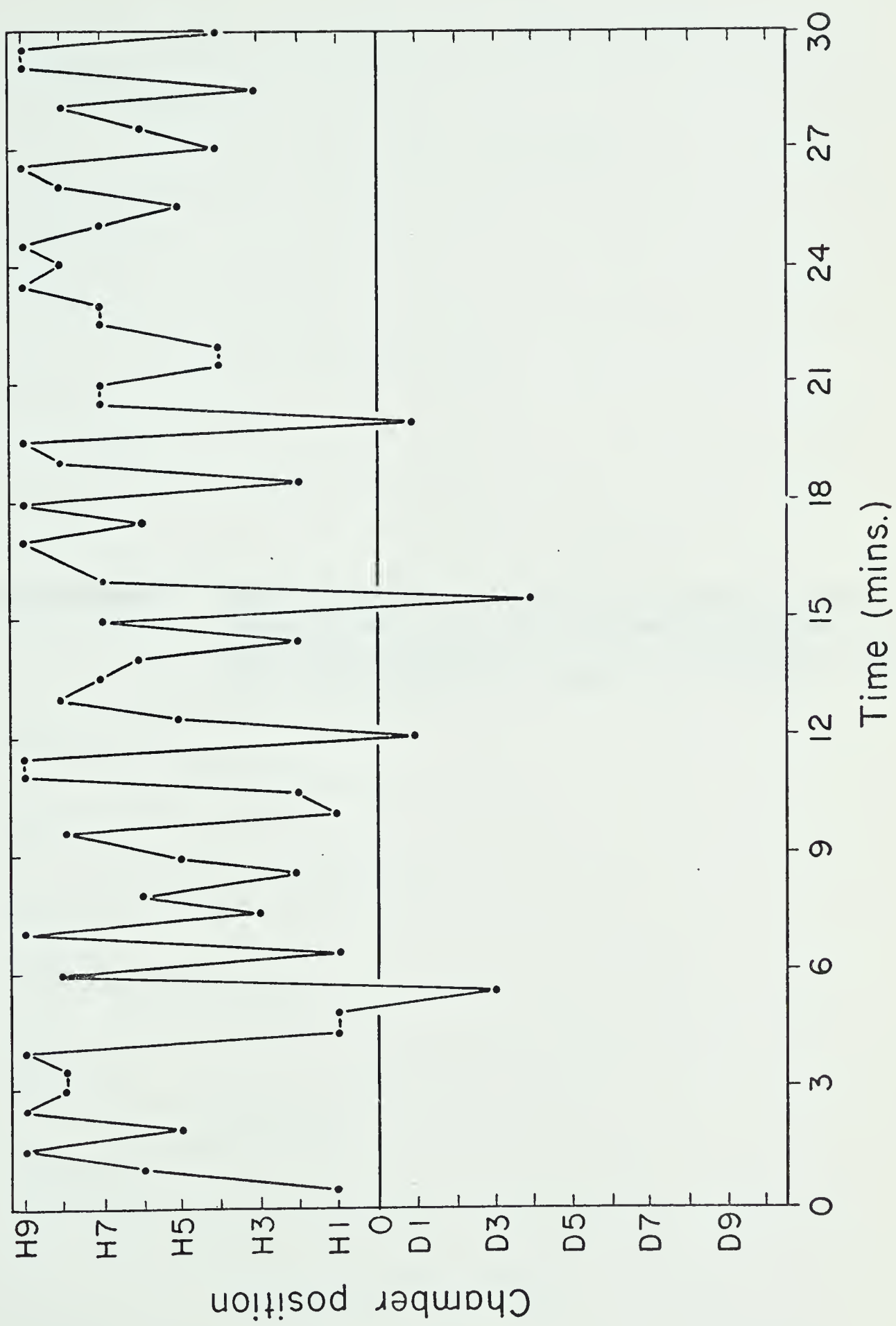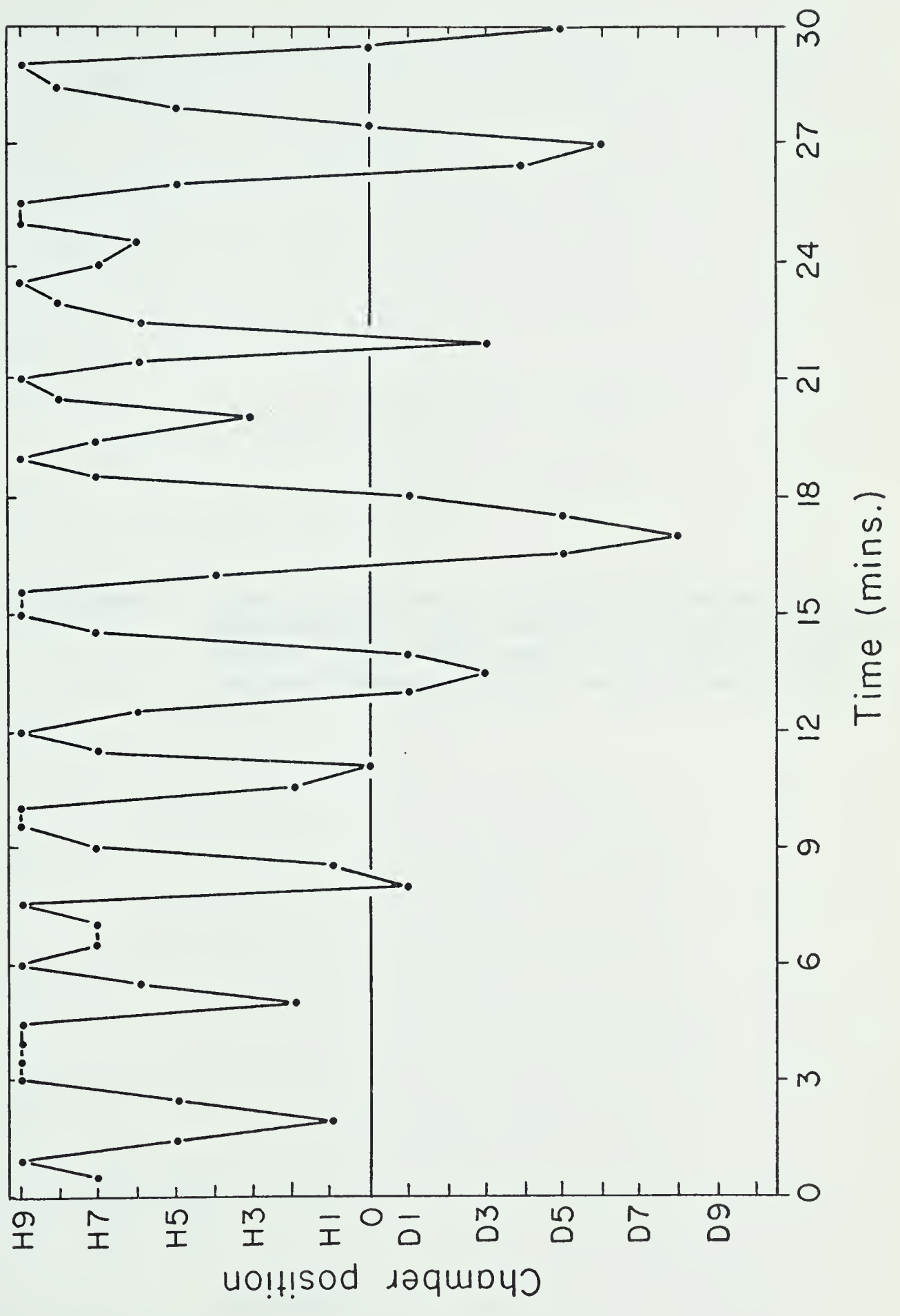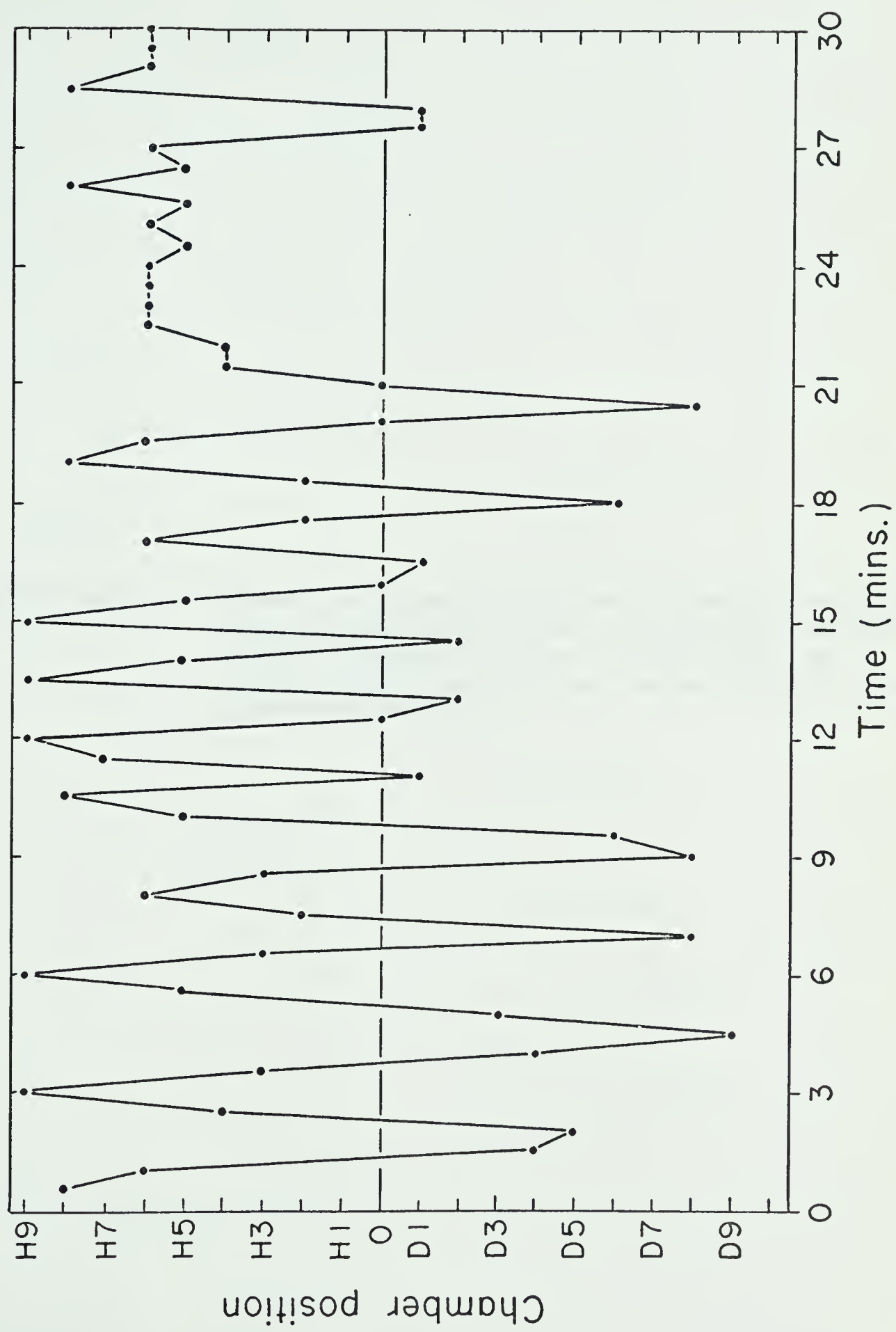                     actual distances.
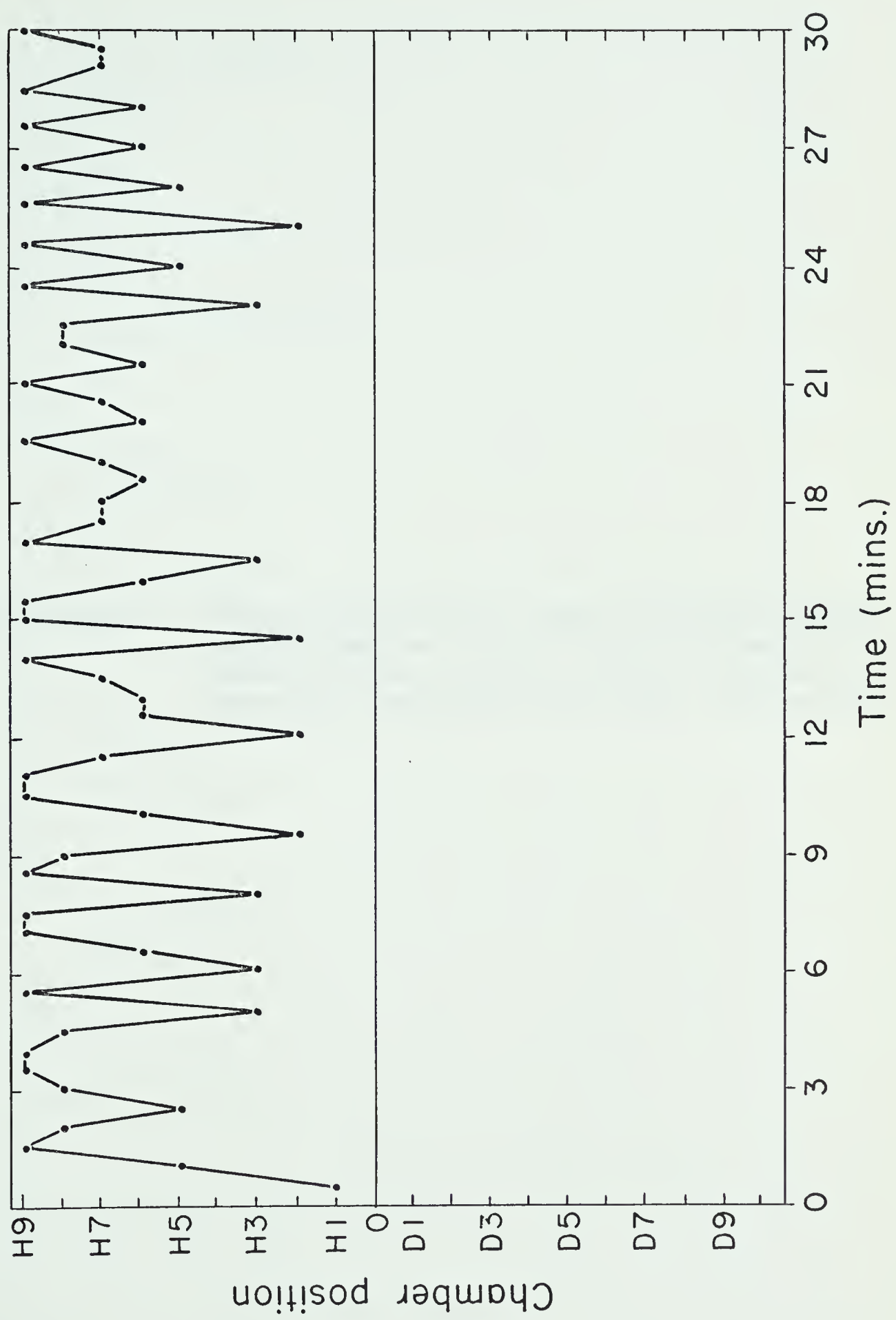
Appendix Figure 2.  Chamber positions of a 100% SW acclimated gradient
tested animal at 0.5 minute intervals showing a
typical response intensity (+86.7%), typical reversal
turns (16), and typical mean speed (10.85 cm/min).
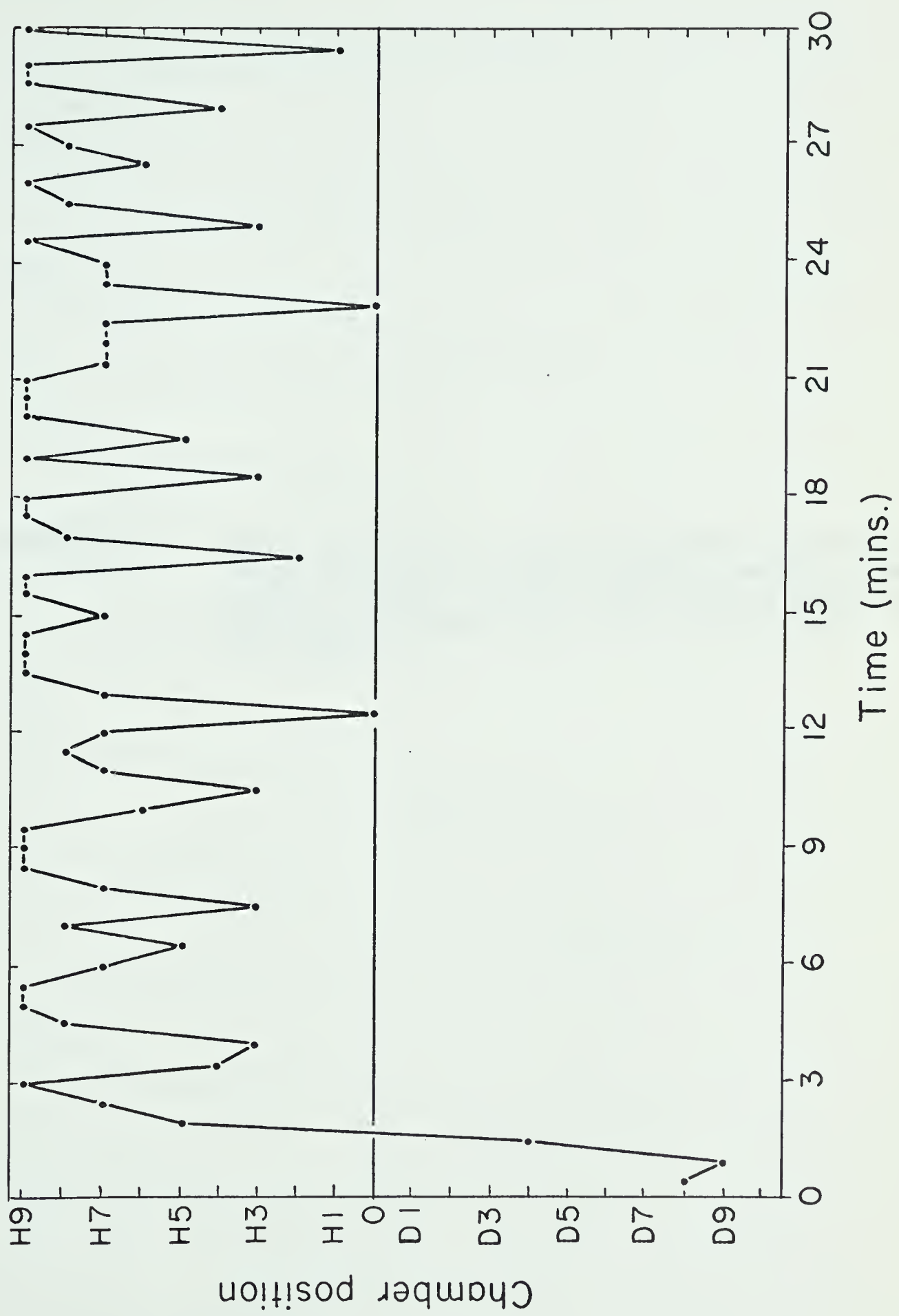
Appendix Figure 3.  Chamber positions of a 100% SW acclimated gradient
                    tested animal at 0.5 minute intervals showing a low
                    response intensity (+53.3%), few reversal turns (6),
                    and typical ordinary turns (10) and mean speed
                    (9.52 cm/min).

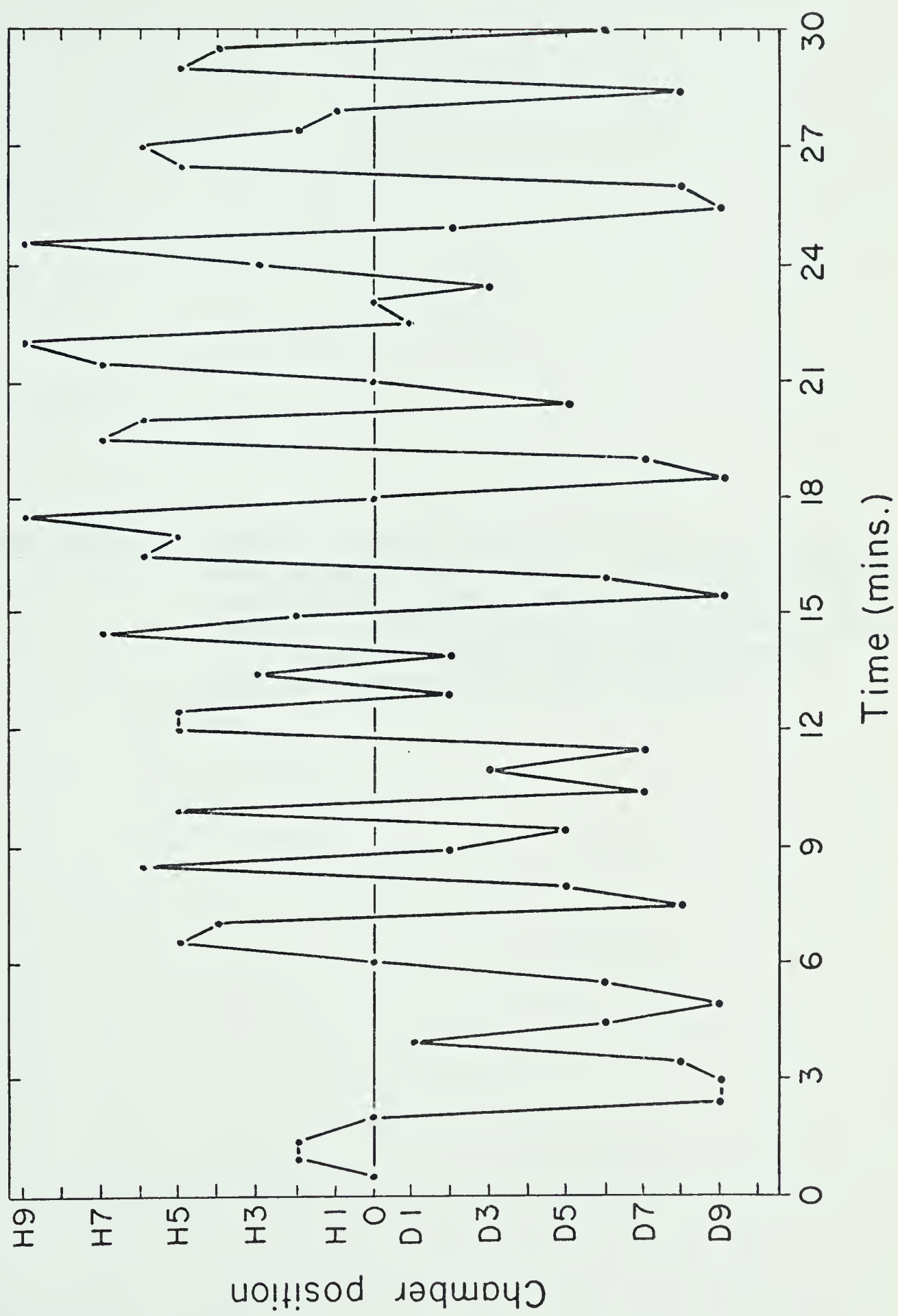Appendix Figure 4.  Chamber positions of a 25% SW acclimated gradient
                    tested animal at 0.5 minute intervals showing a
                    high response intensity (+98.3%), typical reversal
                    turns (16), and a typical mean speed (10.88 cm/min).

Appendix Figure 5.  Chamber positions of a 25% SW acclimated gradient
tested animal at 0.5 minute intervals showing a
typical response intensity (+86.7%), a typical mean
speed (12.85 cm/min), and many ordinary turns (59).

Appendix Figure 6.   Chamber positions of a 25% SW acclimated gradient
                     tested animal at 0.5 minute intervals showing a
                     low response intensity (+55.0%), few reversal turns
                     (8), and a typical mean speed (11.12 cm/min).
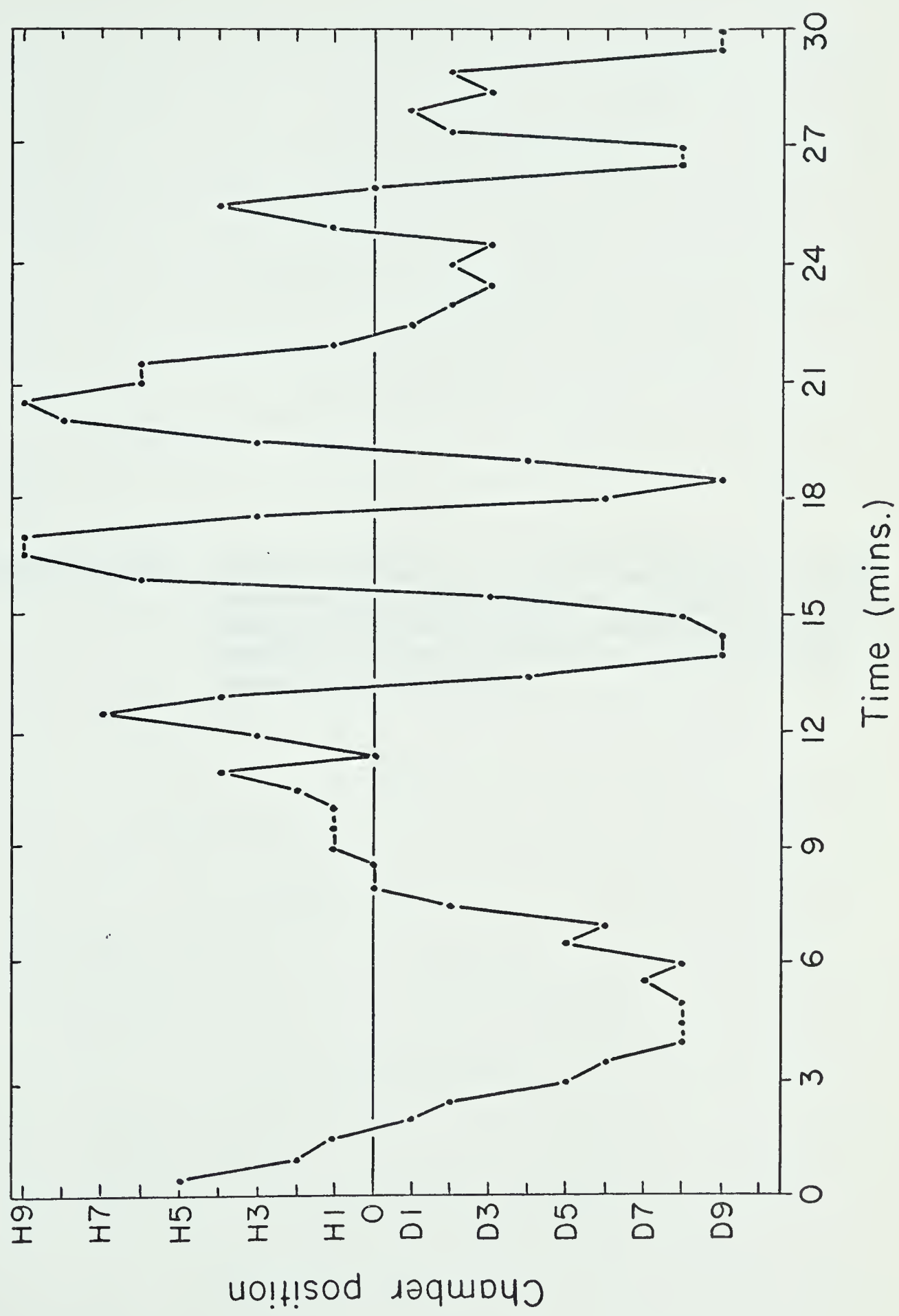
Appendix Figure 7.  Chamber positions of a 25% SW acclimated constant
high humidity tested animal at 0.5 minute intervals
showing a high mean speed (14.24 cm/min), a high
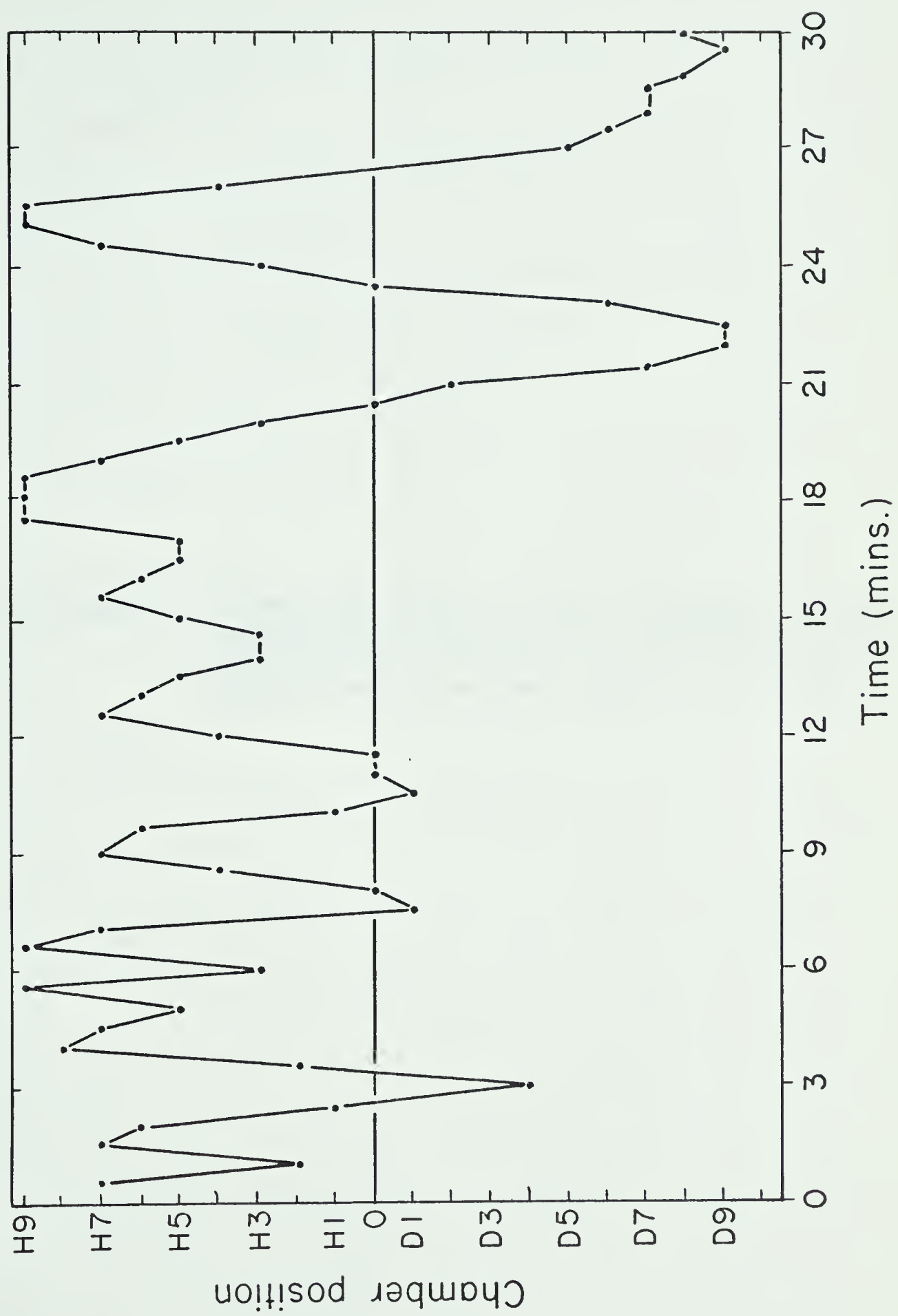response intensity (+40%), and no reversal turns.

Appendix Figure 8.   Chamber positions of a 25% SW acclimated constant
high humidity tested animal at 0.5 minute intervals
showing a typical response intensity (0%), no
reversal turns, many ordinary turns (38) and a mean
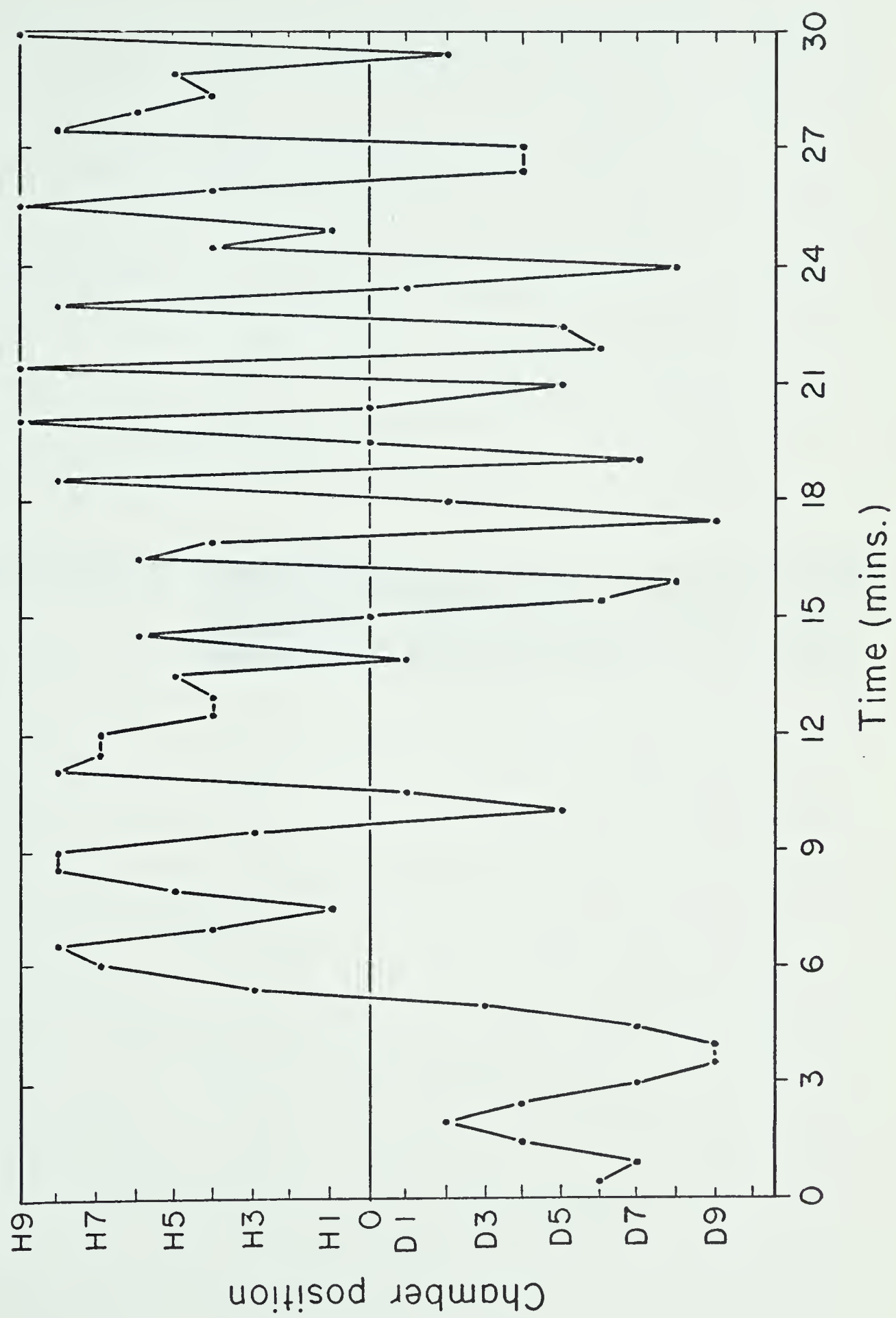speed of 17.36 cm/min.

Appendix Figure 9.  Chamber positions of a 25% SW acclimated constant
high humidity tested animal at 0.5 minute intervals
showing a low response intensity (-16.7%) and a
low mean speed (8.20 cm/min).  Lines between points
in all appendix figures are minimal distances
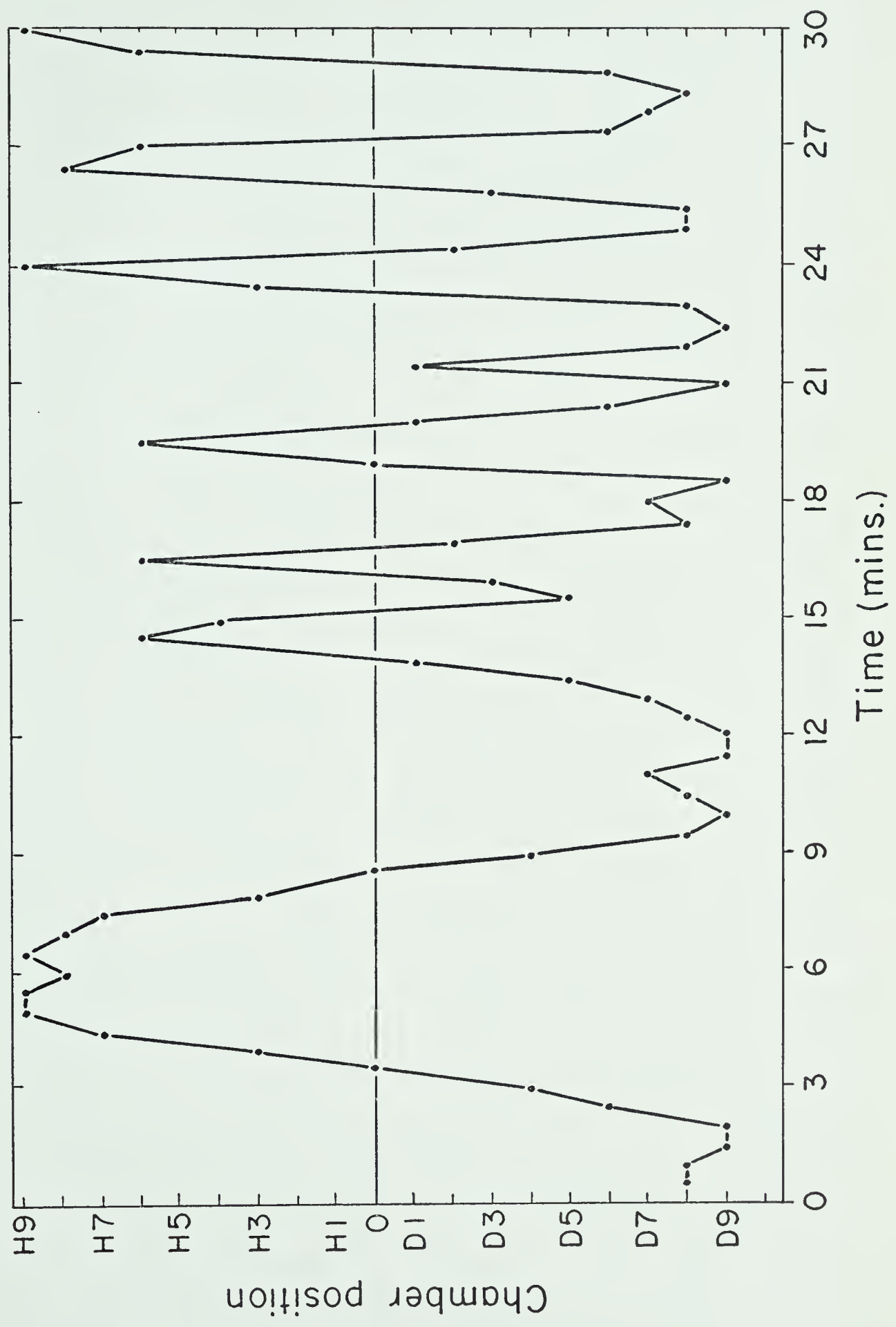between positions, not actual distances.

Appendix Figure 10.  Chamber positions of a 25% SW acclimated constant low humidity tested animal at 0.5 minute intervals showing a low mean speed (8.34 cm/min), a high response intensity (+38.3%), no reversal turns, and few ordinary turns (6).

Appendix Figure 11. Chamber positions of a 25% SW acclimated constant low humidity tested animal at 0.5 minute intervals showing a typical response intensity (+8.3%), no reversal turns, and a mean speed of 17.76 cm/min.

Appendix Figure 12.   Chamber positions of a 25% SW acclimated constant
                       low humidity tested animal at 0.5 minute intervals
                       showing a low response intensity (-31.7%), one
                       reversal turn, and a mean speed of 12.37 cm/min.